

# nature

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## Paying the piper

THE perks of working in the *Nature* office are not extensive. No doubt if the journal were dedicated to the evaluation of stereo equipment or gourmet meals or travel to the tropics we would not lack material benefits, but reporting on advances in science and on science policy hardly leads to obvious free handouts. Can you imagine the Science Research Council's report being distributed with a modest fusion reactor or an 'autonomous house' to keep the press happy? Of course we get the occasional lunch, the odd trip abroad and even an executive jet flight now and then to help us see things in an objective light. We also get an agreeable pile of diaries and calendars (and if Ciba-Geigy read this I'd like them to know how I've enjoyed their calendar of fully dressed high class girls and how I hope I'll get another one this Christmas). And the other day a tiny crate containing a bottle of champagne to help me join in celebrating the launching of a brand new film on embryology the makers of which I can't plug in these columns. But the most interesting perk I've yet had came a few evenings ago.

Bayer AG Leverkusen makes drugs and like every other large company these days a million and one other things also. It also has a symphony orchestra conducted by Rainer Koch and comprised entirely of its own employees. That is unusual, you may say, but not unique; companies keep all sorts of things besides football teams these days. Aren't there famous bands such as Black Dyke Mills Band and Foden Motor Works Band? (I can remember advertisements in the local paper, "Stanton Iron Works requires a tenor euphonium player; office job will be found"). What's new about the Bayer Philharmonic?

I cannot claim to be a music critic, although I do reckon to know a fair bit about orchestras, and not through regurgitating comments from record magazines. Nevertheless it was clear that the Bayer Philharmonic would have stood comparison with the best of amateur orchestras anywhere in the world. For its concert in the Fairfield Hall, Croydon, the company had invited along staff, customers, business associates and, no doubt, rivals ("got anything like that in your company, eh?"). The orchestra started with Vaughan Williams's "The Explorer", film music of the 1950s and a rarity indeed in English concert programmes. Nice as it was as a gesture it didn't really come off. Vaughan Williams is so English and has to be appreciated in the context of green fields, sheep and country pubs; there is something intangibly incongruous in eighty German drug company employees in dinner jackets trying to make the folk songs sing. But enough chauvinism; there was little to fault elsewhere in the programme.

Dinorah Varsi joined the orchestra to perform Chopin's somewhat over-inflated piano concerto in E minor. The orchestra was more in its element here and

it was only the occasional ability all amateur orchestras have to make the piano sound slightly out of tune which detracted from a fine performance. The orchestra concluded the evening with an excellent rendering of Brahms's First Symphony, in which the only weakness of substance was the inability of the woodwind, particularly clarinet and oboe, to make much impression over the top of a fine body of strings.

Now what is the moral of all this, for moral there has to be, even in a Christmas issue? Not that it must take a great deal of 'spare time' to drill so precise a band. Not even that you should go out and buy Bayer products to keep the orchestra in business. Rather that it is a much more intelligent form of patronage of the arts than most companies indulge in at present.

Sponsorship and patronage is under much discussion, because in a period of financial gloom the first thing a business does is economise on its peripherals. There is an obvious tendency to regard sponsorship as advertising and thus to seek to attach the company's name to the best known events both in art and sport. In the short run this probably does little harm and a fair bit of good in keeping admission prices down. But in the long run anything that tends to centralise and to emphasise the cult of the excellent at the expense of personal participation is bad. It is good to know that various London symphony orchestras, theatres and operas are being supported by big business but it would be better to know that big business was equally among the grassroots, raising the levels of competence and enthusiasm of amateurs and young professionals in Scunthorpe, Swansea and Kilmarnock. With modest help, perhaps most intelligently supplemented by the provision of professionals to coach or even write for them, such groups can clearly flourish, and what's more young professionals can be encouraged. It may even have a longer lasting effect on the company image than a once-a-year mention in the programme of a London performance.



AFTER the Franco-Prussian war of 1870-71, it is well known that in many districts in France a new vegetation sprang up, evidently the result of the invasion. It was believed that this vegetation would become acclimatised. It is not so, however, *L'Institut* informs us; at least very few of the species introduced in this way appear likely to continue to flourish on French soil. In the departments of Loiret and Loir-et-Cher, of 163 German species, the half at least have already disappeared, and the surviving species diminish in vigour each year. Scarcely five or six species would appear to manifest any tendency to become acclimatised; these are, according to M. Nouel, *Alyssum incanum*, *Trifolium resupinatum*, *Rapistrum rugosum*, *Melilotus sulcata*, and *Vulpia ligustica*. On the plateau of Bellevue, where in 1871 many strange species were seen, M. Bureau has been able to find only one—*Trifolium resupinatum*. M. Gaudefroy also, who in 1871 and 1872 found many adventitious plants, has been able to collect only two this year—*Ranunculus macrophyllus* and *Linum angustifolium*.

From *Nature*, 11, 135, December 17, 1874.





## Rome: a strategy for survival

Sahel drought: photo by Nick Fogden

*Looking for a strategy to feed the World over the next decade, the World Food Conference put together a comprehensive action pack—but not all its elements looked easily compatible and the conference called on science and research workers to bridge the gap. Robin Sharp who has attended numerous United Nations conferences as a journalist and now heads Oxfam's Public Affairs Unit was in Rome writing for PAN, the highly successful conference newspaper.*

To imagine that giant leaps for mankind could spring directly from a two-week 'talkfest' like the World Food Conference would be to misconstrue the nature and purpose of such events. Indeed it is probably better that the United States, the EEC and the OPEC countries did not come forward at that moment with the billions of dollars being asked for them, because with so much stress laid on the easy-to-grasp, short term crisis, we might all have returned home in a dangerous

state of euphoria. The temptation to call the conference a success would have risked implying that somehow it had 'solved' the problem of how the world is to feed itself in the next 10 years. And nothing could be further from the truth.

The principal function of the Rome meeting was to act as a means of harnessing all available political energy and then channelling it out to power the productive machinery of government, international agencies, research institutes and so on. This it has done and, as far as can be judged at such close range, done to measurably good effect. A momentum has been created; the job now will be to use and sustain it.

Already the dimensions of the immediate crisis are more widely known—some accomplishment in itself. The people of Africa, Asia and Latin America have spent about \$10,000 million in the past year to buy abroad the food they need to live. Now the money has run out, but at the end of the conference they were still short of 7.5 million tons of grain, which would cost about \$2,500 million at market prices. Over the next nine months, reckoned by average

grain consumption in the developing countries, we are talking about the food of 45 million people. And without some drastic intervention, the story over the next 10 years will be the same, with a high rate of compound interest.

Faced with disaster warnings of such magnitude, a world food strategy based on large scale, capital-intensive technological inputs was virtually irresistible: the answers had to be on the same scale as the problems. The potentially serious flaw in this approach is that it depends on how the problems are defined—and they get defined in their 'macro' aspect because that is the only way they are manageable. In fact, the 30-man Food and Agriculture Organisation (FAO) task force which drafted the conference's Action Plan had originally thought of submitting alternative strategies—one based on high technology, the other on labour-intensive schemes utilising intermediate technology and fed in much closer to the grass roots. In the end they tried to blend the two together but the result was something of an oil and water mixture, with a preponderant dose of oil.



To improve the strategy's consistency, Third World delegates and others introduced many amendments underscoring their conviction that the aims of increased food production must be fitted into a pattern of integrated rural development, taking full account of social, cultural and environmental conditions. And perhaps it was because the massive macro schemes often seemed incompatible with the needs of the millions of poor rural workers that the final conference document laid special stress on the need for more research.

In a lengthy resolution from its First Committee, the conference expressed "concern at the inadequate amount of basic and particularly applied research directed to developing new agricultural technology suited to the needs of developing countries, as well as weaknesses in adaptive research, training and extension to achieve more effective transfer and utilisation of both existing and new technology . . ." There is a paucity of trained technical personnel, the resolution added, and much current research "lacks coordination and makes inadequate use of important information already available from research in ecologically comparable regions."

Among its nine proposals on research issues, the conference recommended:

- Rapid establishment of a global network of plant genetic resource centres and the extension of this to animal genetic resources.

- Intensified research by national and international institutions on the application of meteorological information to land-use and other agricultural planning, such as the development of alternative cropping strategies to suit different weather conditions.

- That the FAO/World Bank Consultative Group on International Agricultural Research (CGIAR) should study the feasibility of an international programme on the use of remote sensing techniques in agriculture, including the use of data from the Earth resources satellites.

- That extensive adaptive research programmes be developed, involving testing in farmers' fields the economic and technical viability of new technology and thereby tailoring recommendations to suit specific locations, farming situations and socio-economic conditions.

- That means be found to give the developing countries better access to research equipment and activities, including germ plasm resources.

On specific areas of research, the Action Plan pointed out that the so-called Green Revolution has so far only made a substantial impact on varieties of wheat, maize and rice. It

suggested that barley and triticale—a hybrid between triticum (wheat) and secale cereals (rye) species—were other grains deserving a greater research effort, triticale in particular because of its potentially high yield, high protein content and potential for flourishing on marginal lands. Also, the plan stated, no work is at present supported at international level on oil seeds such as sunflower, safflower and rapeseed, although their oils are important in the diets of many developing countries. Similarly more work is justified on root crops such as cassava, which provide the energy requirements of perhaps 40 million people.

While accepting the need of extended research and development in these fields, less attention was given to the equal need for high-yield varieties able to survive without sophisticated inputs. For it is the cost and scarcity of these essential inputs—chemical fertiliser, diesel fuel for irrigation pumping, petrol for transport—which has suddenly made the first Green Revolution turn sour for many who committed their livelihood to it. And this, in turn, leads to wider questions which the World Food Conference made no attempt to assess. How far has the present crisis been exacerbated by existing large scale rural development projects? How much capital has gone into underused facilities which could have been used to create jobs and therefore food-buying power?

While pondering these dilemmas, there are formidable problems of a more practical kind to be dealt with. For example:

**Development of land and water resources.** The secretariat's programme

called for the improvement of 46 million hectares of existing irrigated lands, the expansion by 1985 of a further 23 million hectares under irrigated agriculture and development of 153 million hectares of new land in rainfed areas. The cost of this programme would, however, amount to \$89,000 million, including about \$30,000 million in foreign exchange—and even before the conference began several delegations had registered their scepticism as to whether resource transfers on this scale could be realistically envisaged or fully absorbed.

**Post-harvest losses.** The importance of improved food storage received only a passing mention in the final resolutions but figures cited in the Action Plan illustrate the need for better facilities at all levels of distribution. Post-harvest losses in cereals, as a result of mildews and fungus diseases, rodents and insects, are estimated at 5 to 10% but up to 40% in exceptional cases. Losses of 30 to 40% are common in perishable fruit and vegetables. Insects consume the most nutritious part of the grain, leading to deterioration in its calorific, protein and vitamin contents; and damaged grain absorbs moisture, encouraging the growth of harmful micro-organisms.

**Nutrition.** Information on food consumption patterns and on their consequences for the nutrition and health status for the majority of people in developing countries is insufficient, said the 15-joint resolution on this subject. Improved knowledge about how to prevent malnutrition through better use of available food resources, including human milk, is essential. The conference called on governments to explore the "desirability and feasibility of meeting nutrient deficiencies, through fortification of staples or other widely consumed foods, with amino acids, protein concentrates, vitamins and minerals." It also recommended the establishment of a global nutrition surveillance system to monitor, *inter alia*, the condition of disadvantaged groups, and an inventory by the FAO of vegetable food resources other than cereals, including roots, tubers, legumes and "also those from unconventional sources."

Now these and the many other recommendations of the Rome conference are in the hands of the United Nations, its various specialised agencies and individual governments, waiting to be implemented.

Ultimately, as the conference Secretary-General, Mr Sayed Marei, phrased it, "judgment on the success or failure of this conference is going to be made by hungry men and women in Africa or Asia, or by a hungry child . . ." But how long will they have to wait? □

Botswana food programme : FAO photo





THE REPORT of the work of Dr J. W. King and his colleagues at the Appleton Laboratory (252, 2; 1974) recalls articles contributed to *Nature* almost a century ago by William Stanley Jevons, economist and statistician, in which he sought to evidence the relation between sunspots and commercial crises (19, 33; 1878 and 19, 588; 1879).

Jevons's early education was in mathematics, chemistry and botany. He later developed an interest in, and wrote widely on, meteorology. This scientific training influenced his approach to the study of economics. In *Letters and Journal of W. Stanley Jevons*, edited by his wife (Macmillan, London, 1886) we read:

'He published in Waugh's *Australian Almanac* for 1859, "Some Data concerning the Climate of Australia and New Zealand", a paper over fifty pages in length, which is best described by his closing words: "My object has been to present in an available form such accurate numerical data as are attainable, and secondly, to group together general information as to the winds, rains, rivers, floods, the geographical features of the country, and the meteorological circumstances of this part of the globe, so as to show what remarkable problems have to be solved, and what interesting connections of cause and effect may ultimately be traced and proved."

The significance of his method was that, translated into the context of 'political economy', it represented a new approach to the investigation of economic phenomena. In a paper read to the meeting of the British Association at Cambridge in 1862, "On the Study of Periodic Commercial Fluctuations, with five diagrams", Jevons wrote:

"It seems necessary, then, that all commercial fluctuations should be investigated according to the same scientific methods with which we are familiar in other complicated sciences, such especially as meteorology and terrestrial magnetism."

In 1862, when this was written, Jevons was concerned with seasonal changes and not with the longer swings of the trade cycle; and his interest in meteorology had not led him to the theory of solar variation to explain these larger swings. His famous conclusions on the underlying causes of the trade cycle were advanced in two papers presented to the British Association in 1875 and 1878.

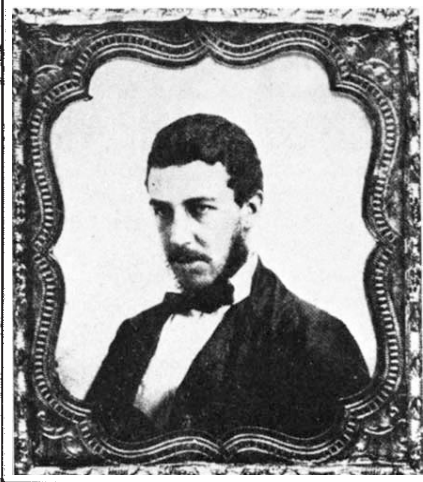
The fate of the first paper, "The Solar Period and the Price of Corn" is recounted in J. M. Keynes's *Essays in Biography* (Mercury Books, London, 1961):

'Thorold Rogers' "History of Agriculture and Prices in England", which

began to appear in 1866, provided Jevons with material for analysing wheat prices over a long period. The commercial crises in his own lifetime had occurred at intervals of ten or eleven years: 1825, 1836-39, 1847, 1857, 1866. Might there not be a connection between these things? "I am aware," Jevons concluded, "that speculations of this kind may seem somewhat far-fetched and finely-wrought; but financial collapses have recurred with such approach to regularity in the last fifty years, that either this or some other explanation is needed." Nevertheless he soon repented of publishing

## Sunspots and the business cycle

*J. R. Sparkes, lecturer in economics at the University of Bradford, looks at the work of W. S. Jevons (below), who tried to relate sunspot activity and commercial crises a hundred years ago.*



what was no better than a bright idea. "Subsequent enquiry convinced me that my figures would not support the conclusion I derived from them, and I withdrew the paper from publication".

But Jevons still did not regard the enquiry as worthless and was encouraged to discover, after withdrawing his paper, that Sir William Herschel had tried as early as 1801, although with negative results, to explain the variation in the price of corn by the decennial variation of sunspots. Thus by 1877 he was working at the subject again and was more convinced than ever that there was some connection between sunspots and the price of corn. In a letter to Professor J. d'Aulnis de Bourouill in February 1878 he wrote, concerning the years when there were commercial crises, "The periodi-

city is remarkable, and the average length of the period is somewhere about 10.3 years, so nearly the same as the sunspot period that there can hardly be a doubt about the connection of cause and effect."

Jevons's ideas were spelt out in a second paper to the British Association, "The Periodicity of Commercial Crises and its Physical Explanation", and the first of two articles contributed to *Nature*. Although still concerned about the evidence on which his theory of crises was based, he had great confidence in its substantial truth.

Keynes, in his essay, listed the new discoveries which were his excuse for returning to his theory:

(1) He had succeeded in carrying back the history of commercial crises at ten or eleven-year intervals almost to the beginning of the eighteenth century.

(2) He was now advised by his astronomical friends that the solar period was not 11.1 years, as he had previously supposed, but 10.45 years, which fitted his series of commercial crises much better.

(3) He now abandoned European harvests, the price statistics for which yielded negative results, as the intermediary through which sunspots affected business, in favour of Indian harvests, which, he argued, transmitted prosperity to Europe through the greater margin of purchasing power available to the Indian peasant for buying imported goods.

But apart from the coincidence of the periodicity of the commercial crises and the solar period, the causal link was never much stronger than a personal conviction that the decennial crises depended on meteorological variations of similar periodicity. His 1875 evidence, which depended on European harvests, was overturned in 1878 in favour of fluctuations in foreign trade resulting from cyclical crop changes in India and elsewhere. But even then the timing of the relationship was such as almost to suggest that the effect preceded its cause.

Later empirical work has largely discredited the theory that there is a link between business cycles and a 10 to 11-year cycle in sunspot activity. Business cycles have recurred at shorter intervals than 10 or 11 years, and in spite of ingenious interpretations of harvest statistics over the years, it is now generally agreed that such an explanation could never completely account for the business cycle. Keynes's assessment is summed up as follows: "Jevons's notion, that meteorological phenomena play a part in harvest fluctuations and that harvest fluctuations play a part (though more important formerly than today) in the trade cycle, is not to be lightly dismissed." □

# international news

As part of a desperate bid to get a grip on inflation, President Ford is proposing to prune nearly \$5,000 million from federal expenditures during the 1975 fiscal year, which has only seven more months to run. Welfare, health and veterans' benefit bear the brunt of the cuts, but biomedical research, space research and several nuclear power programmes are also in the running for some radical surgery.

Ford's proposals came in three chief parts. First, he asked Congress to take the knife to the Appropriations Bill for the Department of Health, Education and Welfare (HEW), which was then in the final stages of passage through the Congressional mill. Among the cuts Ford requested was a reduction of \$112 million in the budget for the National Institute of Health (NIH), a move which he said would reduce expenditures on new grants and contracts by 25% and cut continuation grants by 5%.

Such surgery, he maintained, "could be accomplished without seriously altering the momentum in biomedical research achieved by sustained and substantial growth in the National Institutes of Health in recent years", but since the NIH was hit hard by cut-backs in 1973 and received only a modest budget increase this year, few biomedical scientists will agree with that sanguine prediction. Congress, in

## Cutting budgets to fight inflation

by Colin Norman, Washington

any case, responded to the request by passing the HEW Appropriations Bill last week with a massive increase of \$256 million in the NIH's budget instead of a decrease of \$112 million, thereby putting the ball firmly back in Ford's court.

The Appropriations Bill would step up spending in every NIH institute, with the National Cancer Institute topping the list with a budget of \$692 million—an increase of \$164 million over last year. The National Heart and Lung Institute would also receive a massive infusion of funds, amounting to \$324 million, and the entire NIH budget would climb to \$2,090 million. The Appropriations Bill additionally provides a pot of money for biomedical training, which the Administration has been trying unsuccessfully to decrease.

Ford, it seems, has two options open if he is determined to press his case over the HEW budget. Either he can veto the Appropriations Bill to try to force Congress to reduce the amounts, or he could sign it and later propose that some of the increases be deferred until next year.

Finally, Ford is recommending that money which Congress has already allocated to some programmes be spent next year instead of this year. Among the programmes he has selected for such a stretch-out are several nuclear power projects, some space research and development, and solar and geothermal energy research supported by the National Science Foundation.

He is suggesting, for example, that \$80 million in the budget of the Atomic Energy Commission be deferred, including \$8 million earmarked for the breeder reactor, \$8 million for controlled thermonuclear fusion, \$4 million for environmental research and safety, \$6.7 million for development of hardware for the high temperature gas cooled reactor, and another \$13 million for equipment needed for the fusion programme. Although Ford maintains that the delays will have little lasting impact on the nuclear power programme, those proposals provide an important indication that the Administration may be losing some of its overriding enthusiasm for nuclear energy.

As for the space programme, Ford is requesting authority to withhold until next year some \$20 million earmarked for the joint USA-USSR docking mission. He also wants to defer expenditure of \$16 million for several space science and applications projects; and \$36 million from NASA. □

THE six-year-old collaboration arrangement between the World Health Organisation (WHO) and the government of India, involving a research project on genetic control of mosquitoes, is due to expire in June next year. Following a controversy in India (*Nature*, September 20) over the American defence establishment's apparent interest and role in these projects, the Indian government has been rethinking.

It is almost certain that the WHO will ask for an extension of its agreement with India, because some of the project's planned experiments will not have been completed by June 1975. (A big experiment aimed at controlling *aedes aegypti* genetically is scheduled to commence early next year.) Should there be an extension of the project, it will come only after the present agreement with the WHO has been revised with new or amended provisions. Among other things, India will, in all probability, insist that the

Genetic Control of Mosquito Unit (GCMU) be placed under the overall charge of the Director-General of the Indian Council of Medical Research (ICMR) and that all project leaders be appointed only with the specific approval of the government of India. The Indian government will also very carefully examine various provisions of the WHO agreement with the United States government, to ensure that all the necessary safeguards are provided in its revised agreement with the WHO—that is, in case it decides to extend the latter's mosquito research project beyond next June.

According to a note prepared by the Union Health Ministry on the GCM project and placed before the Lok Sabha (lower house of the Parliament), a recent joint meeting of the expert committee on virus and arthropod-borne diseases and geneticists from the expert committee on human genetics, immunology and allergy, has favoured

the creation of a separate 'monitoring body' with a membership drawn from those not actually engaged in the project. This body, in close co-operation with the concerned GCMU staff, will monitor the effect and impact of all future releases of 'genetically manipulated' mosquitoes at all stages of the release operation. The meeting also recognised that consideration of the possible future lines of development for the ICMR-WHO project was the legitimate responsibility of none other than the GCMU's own project committee.

Meanwhile, at a meeting held last month of the subcommittee set up by the ICMR's governing body to review the administrative and technical control of the GCMU, it was felt that the project leader should be asked by the Director-General (of the ICMR) to submit reports to him every 15 or 30 days about the work done in the unit.

from Narender K. Sehgal



CHANGES in the environment, for better and worse, are reviewed in the Fourth Report of the Royal Commission on Environmental Pollution, published last week (Cmnd 5780, HMSO, £1.10).

Little progress seems to have been made on old problems like the control of pollution in estuaries, but the situation with others, such as the use of persistent insecticides in agriculture, seems to be improving and there are new problems, such as noise, which the commission has picked out as a growing threat. Twenty-nine million people in urban areas in Britain are expected to be affected by 1980 through the growth of traffic noise above acceptable limits.

Inevitably there is concern that the environment could suffer unduly in the present financial climate, and it will be argued that action on environmental projects that cannot be evaluated in strict economic terms should be deferred. The commission comes out strongly against such arguments and feels that they should be "strongly resisted". "At the very least there should be permitted no further deterioration in environmental quality", it declares.

There is a useful section on 'who does what' setting out the structure of pollution control in the United Kingdom and the responsibilities of local authorities and the special statutory bodies. The commission also notes the increasing influence that bodies such as the European Economic Community are having and are likely to have on British pollution legislation. A section

showing the interaction between United Kingdom legislation and international and European recommendations seems a must for the next report.

● The cuts in British defence spending will reduce military research and development expenditure by 10%. The detailed incidence of reductions is still

## Round Britain

largely a matter of negotiation in defence establishments and is not expected to be known for several months. Talk about increased coordination of research and development across NATO to save by avoiding duplication of expensive projects is largely misguided; collaboration is already fairly extensive, so the cuts cannot be confined to rationalisation. The Polaris programme remains untouched.

● THE creation of new, tenured posts in astronomy at any university is an event so rare these days as to merit attention from the astronomical community around the world. Just such a situation has occurred in Cardiff, where the Department of Applied Mathematics and Astronomy at University College recently moved to a new building and expanded its astronomy interests, under the guidance of Professor N. C. Wickramasinghe.

The atmosphere of the group is best summed up as one of youthful enthusiasm combined with a good deal of astronomical and mathematical ability. Some of the more conventional studies

include aspects of the formation of stars and galaxies; at the other extreme one member of the team is working on aspects of the Hoyle-Narlikar theory of gravitation, and another is investigating problems in quantum gravity and relativity, which include the possibility of adding time to the list of quantum variable parameters.

It is hardly surprising that Wickramasinghe is full of praise for the way in which both the Science Research Council and University College, Cardiff, are supporting astronomy. In an interesting echo of remarks made recently by some other senior astronomers in the United Kingdom, he mentions that there is great interest in astronomy at undergraduate level, and says that there has already been an increase in enrolments in the mathematics department, partly as a result of the astronomy courses now offered.

● This year's crop of medals from the Royal Society conforms to the pattern of the past twenty years. Everything goes to Britons and all except the two specifically for achievement in industry (the Esso Medal is a newcomer this year) go to male Fellows. Sir Alan Hodgkin in his Presidential Address is slightly defensive about the quality of the medallists in the light of "some mild sniping from *Private Eye*". The quality we don't doubt, but the narrow national perspective goes against the intentions of the donors, some of whom prescribed specifically that the award was to be made regardless of nationality. Or sex.

THE flight of Soyuz 16 (December 2-8, 1974) was specifically announced to have been a preparation for the joint Soyuz-Apollo programme scheduled for 1975. Observers of the Soviet space programme have interpreted recent Soyuz flights (including that of Soyuz 15 which somewhat coyly manoeuvred about the Salyut 3 space station, without actually effecting a link-up) as being a preparation for the joint programme.

The official purpose of the flight was described by the TASS agency as the "testing of on-board systems of the Soyuz craft, which have been modernised in accordance with the requirements of the joint flight, the carrying out of scientific and scientific-technical investigations, and also observation and photography of individual sections of the Earth's surface in order to obtain data for the solution of problems of the national economy." Leaving aside the final clause (which is included in all descriptions of Soviet

## Preparing for that space link-up

from Vera Rich, London

space missions as a sop to the economic planners) perhaps the most interesting word in the whole release is "modernised"—which, to the student of Soviet semantics displays a refreshing honesty. At one time the word would surely have been 'adapted' and the implication would have been that in any international project it would not be the Soviet side which would have had to 'modernise' to meet the needs of the other.

The experiments carried out by Soyuz 16 relate, in fact, both to the details of the link-up and to the requirements of the on-board systems. The life-support systems were tested and the medical checks made with a cabin pressure of 540 mm Hg, which suggests a working atmosphere rich in

oxygen. Hitherto, the Soviet programme has preferred a natural atmospheric mix, so this implies something of a compromise with US standards.

Preparations for the link-up included an orbital transfer, on December 3, into a circular orbit at a height of 225 km and inclination 51.8°, described as "similar" to the one the Soyuz craft will have to adopt in the joint mission. In the course of the 32nd, 38th, and 48th orbits, the new link-up systems, "created in accordance with the requirements of the 1975 joint mission" were tested *in toto* and also unit by unit, the tests being monitored by cosmonauts Filipchenko and Rukavishnikov themselves, as well as by ground control. Simulated link-up tests were carried out satisfactorily.

The programme also included a number of astrophysical and biological experiments, the latter ranging from the exchange of micro-organisms to the effect of prolonged illumination on the cosmonauts. □

# correspondence

## Entry forbidden

SIR,—This year I had planned to attend the Ninth International Meeting of FEBS (the Federation of European Biochemical Societies) in Hungary. I am a scientist, I paid my registration fees and I even had flights and accommodation arranged; but I was barred on grounds of nationality. I am Israeli!

I suppose the word international has a different meaning in Hungary.

A PhD student at King's College, London, and a member of the British Biochemical Society, I acted in accordance with the instructions in the booklet issued by the Hungarian organisers, "... Participants from countries that do not have diplomatic relations with Hungary should apply for a visa to a General Consulate of Hungary in a European country they find most convenient", and applied for a visa from London.

Even though they had four weeks to issue the visa and in spite of a special request from Professor H. R. V. Arnstein, Secretary of FEBS, they kept me waiting until the very last moment. It was intimated that I could pick up the visa when I stopped in Vienna on my way to the conference. But when I arrived at the Embassy in Vienna, three days before the conference, I received a rude and cold reception. After keeping me waiting for half a morning, they told me I was on a "black list" and forbidden entry to Hungary.

This left me in Vienna, holding worthless train and hotel reservations, wondering about the purpose of international conferences.

I would say to all organisers that if their conferences are to be truly international, they should choose countries which foster this spirit, not, for example, Hungary.

Yours faithfully,

S. PELLER

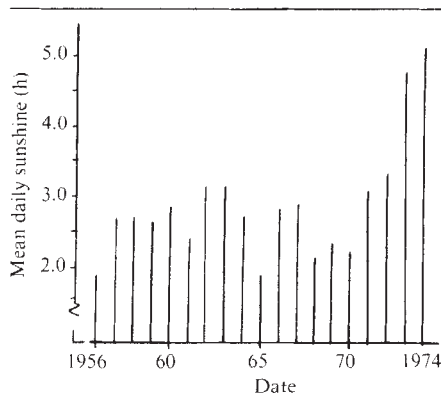
King's College,  
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## Cameroon climate

SIR,—The catastrophic effects on humanity of the major climatological fluctuation which has caused so much concern in the Sahel zone of Africa may have overshadowed other smaller changes in the meteorological records recently obtained in adjacent areas.

Sunshine records obtained during the past 19 years in Yaounde and published by the National Meteorological

Bureau of the Cameroon indicate, perhaps, a considerable shift in the distribution, and thus the total amount, of daily insolation during some agriculturally important months of the year. For example, the mean daily number of sunshine hours for July in the years 1959–74 is illustrated in the accompanying graph. The difference in the years



1973 and 1974 by comparison with the previous distribution of approximately 2 to 3 hours a day could, if confirmed by subsequent records, possibly affect the agricultural productivity of the region, which relies heavily on cocoa, a crop noted for the complex relationships between growth/yield and the shade factor.

The way in which the production of dry matter depends on energy provided by insolation is well known and if the radiation available for agricultural production has altered over a wide area, the aid programmes planned by donor agencies may need revision.

J. DANCER

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## Asbestos and health

SIR,—In Peter J. Smith's article (October 18) the point is well made that scientists should be more concerned with the practicalities of occupational health. We think it a pity, however, that he should have selected asbestos as an example of a health problem which scientists have ignored, since more scientific effort has in fact been expended on this problem than on most other similar problems in recent years, as the literature testifies.

It is not true that "asbestosis is taking an increasing toll" in this country. The number of new cases diagnosed may be on a higher plateau now than it

was in the 1950s, reflecting factory conditions long ago and the increased usage of asbestos in an uncontrolled way in, for example, insulation, but since reaching a peak of 168 in 1967 there has been no further increase. The average since then has been 139 per year. We have every confidence that stringent controls associated with the Asbestos Regulations 1969, which apply to all asbestos work, will in coming years reduce these numbers to a very low level indeed. Indeed they are intended to eliminate them altogether.

The standards associated with the controls have a scientific base for which scientists working for government departments, the Medical Research Council, the British Occupational Hygiene Society and the industry-sponsored Asbestosis Research Council deserve full credit.

Yours faithfully,

W. P. HOWARD

The Asbestos Information Committee,  
London W1, UK

## Academic consultancy

SIR,—Your editorial "Academics in the Boardroom" (November 22) implies that consultancy by university staff is something to be encouraged, whereas in fact it is something to be deplored.

Professors and lecturers at universities are in receipt of a full salary for a full-time job of teaching and research, and none of them has any right to use any part of that time in consultancy to anyone, whether industrial firm or individual, or even government department or agency, for the benefit of his client or himself.

In addition no member of university staff has the right to use apparatus and facilities provided at public expense for the purposes of teaching and research, for the benefit of any organisation or for his own benefit.

It is time that the Department of Education and Science clamped down on such use of public funds to subsidise such consultancy, which has forced so many independent consultants out of business. How can any independent consultant possibly compete with the university member who does not have to pay for his equipment and facilities or premises, and may even use students as unpaid staff?

Yours faithfully,

H. A. COOK

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# news and views

## Excuse me, your slip isn't showing

THE inexorable motion of plates relative to each other at the Earth's surface leaves behind a magnetic record at ocean ridges where new plate is formed. Much of the early work on plate tectonics was devoted to using this record together with a knowledge of geomagnetic field reversals to measure relative plate motions. By 1970 most geophysicists were agreed on the values for inter-plate velocities, typically a few centimetres per year. The motion was obviously manifested, in fits and starts, by earthquakes. But a most important question remained—did the rate determined as an average over several million years tally with the present rate determined from the cumulative displacement of successive earthquakes? For if not, either the movement had a cyclic character, or some of the strain build-up was relieved by non-violent processes such as creep on the fault plane. The relevance of all this to earthquake prediction efforts is obvious.

In 1971, Davies and Brune (*Nature phys. Sci.*, **229**, 101–7) made a major contribution to resolving this question. The only parameter of earthquakes which has been reliably measured over the last seventy years is magnitude. The logarithm of the amplitude of the radiated surface waves (corrected for distance), or surface wave magnitude,  $M_s$ , is a rough and ready measure of the slip on the fault plane once some assumptions have been made about the dimensions of the fault surface. These assumptions, it is widely admitted, are fairly major. Davies and Brune considered the magnitudes of very large earthquakes on all plate bound-

aries and concluded that the inferred displacements over the last seventy years agreed rather well with plate tectonics predictions. Thus it was possible to deduce from their results that for most plate boundaries the part that was played by, for example, creep was certainly not a dominant one. The Mediterranean region did not immediately fit into this scheme. For one thing the tectonics of the area is immensely complex so it is not easy to talk about a small number of plate boundaries. For another, the area is not highly seismic so the data are fewer (the frequent damaging earthquakes in the region result more from the proximity of people to faults than from very high magnitude events). The area clearly warranted a special study and this has now been done by North who reports in this week's *Nature* (page 560).

He comes up with a most interesting conclusion. When similar techniques to those of Davies and Brune are applied in the Mediterranean, the movement on most plate boundaries inferred from earthquake magnitude is very much less than plate tectonics would have it. Either strain is being built up throughout most of the region (and nowhere else in the world) on various types of fault—or strain is being relieved in the Mediterranean area by other processes than major earthquakes—and again this is not happening elsewhere in the world. It is a provocative and important result and should stimulate more research both in the field and in the laboratory.

DAVID DAVIES

## Switching off symmetry breaking

THE elementary particles nearly obey a number of simple and elegant symmetry rules. Many useful approximate calculations can be made by assuming that the symmetries are perfect—for instance by assuming that the decays of  $K^0$  mesons are well behaved when all the particles in the decay process are regarded as travelling backwards in time (time-reversal symmetry), or by assuming that the proton and neutron have the same interactions as the lambda, sigma and xi hyperons. Recently a great deal of progress has been made in building field theories of the elementary particles, such as the unified weak and electromagnetic theories which have been supported by the discovery of 'neutral weak currents' (see *Nature*, **245**, 119; 1973 and **250**, 186; 1974). These new theories incorporate symmetry breaking as a 'spontaneous' effect, drawing on successful theories of low-energy solid-state phenomena such as superconductivity which is also caused by 'spontaneous' symmetry breaking.

When a superconductor with zero resistance is placed in a high magnetic field, above some critical strength, it reverts to being a normal conductor. The theory of this transition has been taken as a model by Salam and Strathdee (this issue of *Nature*, page 569). They show that the same kind of behaviour could occur for a number

of the symmetry-breaking effects in the weak interactions of elementary particles. For instance, there may be a critical magnetic field which would turn off the time-reversal asymmetry in  $K^0$  decay. They estimate this field to be between  $10^9$  and  $10^{14}$  gauss— $10^4$  or more times what can be achieved in a practical apparatus. Another example they give is that the  $\beta$  decay of the lambda hyperon may be turned off at  $10^{16}$  gauss.

Although such field strengths may be difficult to achieve in the laboratory—partly because of the critical field effect itself in superconductivity which limits the strength of our magnets—they may already exist inside pulsars. If so, it may be possible to devise ways of detecting the effects of an absence of symmetry breaking. Another way of achieving high field densities may be through laser compression, using similar techniques to those being developed in research on thermonuclear fusion.

Even if we cannot achieve the predicted fields, they urge that experiments should be started to look for this kind of effect. Their current calculations are so model-dependent that they cannot rule out new effects at fields as low as  $10^6$  gauss.

D. J. MILLER

# Chromosomes and malignancy

ABNORMAL chromosomes are a regular feature of tumour cells. Non-transformed cells rarely show chromosomal abnormality unless obtained from developmentally defective individuals, many of whom show an increased predisposition to cancer. Altered chromosomes therefore either predispose oncogenic change or accompany it. Most cancer-producing agents cause chromosome damage, which may therefore be an important aspect of their potency for tumour induction. But the majority of such damaged cells are inviable, a few are transformed and only a proportion of these may have malignant properties. These wide ranging manifestations of the transformed state have attracted the attention of many workers seeking some explanation in terms of the accompanying chromosomal alterations.

Sachs and his group (Hitotsumachi, Rabinowitz and Sachs, *Nature*, **231**, 511; 1971) have shown that the *in vitro* propagation of polyoma transformed cells may be accompanied by loss of malignant characteristics and the appearance of new groups of chromosomes which are suggested to carry suppressors of malignancy; the balance of these chromosomes in the nucleus is supposed to be an important feature of its control. Harris and his coworkers (Klein, Bregula, Weiner and Harris, *J. Cell Sci.*, **8**, 659; 1971; Weiner, Klein and Harris, *J. Cell Sci.*, **12**, 253; 1973) have fused together cells with and without malignant properties. The result of such an experiment is usually suppression of malignancy, followed by its restoration after the loss of chromosomes from the normal, non-malignant, parent. Fusing together two malignant cells however usually resulted in a malignant cell without the necessity for chromosomal loss. In certain cases the latter experiment resulted in loss of malignancy (Harris, *Proc. R. Soc.*, **B179**, 1; 1971), all of which leads Harris to stress the role of individual chromosomes which carry malignancy suppressors as the important feature of its control. Both groups invoke chromosomally based factors as the essential elements, although in neither case are particular chromosomes designated as the carriers.

In this issue of *Nature* however (page 610) Codish and Paul report the reversible appearance of a specific chromosome which suppresses malignancy in a chemically induced tumour. When present in two copies, both malignancy and antigenicity are suppressed, but both are restored when only a single copy is present. The extra chromosome is thought to originate from the fusion of part of a normal number 19 mouse chromosome with an unaltered number 7. Since the normal elements of this marker chromosome are also present in the cells, it seems possible that the suppression involves a position effect following fusion of the two normal chromosome segments, although this is not suggested by the authors. There seemed to be no other consistent correlation with suppression of malignancy.

A somewhat similar mechanism, resulting in the acquisition of malignancy rather than its suppression, is suggested in the case of the now classical examples of human tumours: chronic myeloid leukaemia in which 90% of cells exhibit a loss of most of the long arm from chromosome 22 (Caspersson, Gahrton and Lindsten, *Exp. Cell. Res.*, **63**, 238; 1970) and the acquisition of a similar fragment by the number 9 chromosome (Rowley, *Nature*, **243**, 290; 1973); and meningiomas in which there is also loss of part of the number 22 chromosome (Zankl and Zang, *Humangenetik*, **14**, 167; 1972). Other cases of specific abnormalities include the number 14 chromosome with an additional terminal fluorescent band in tumours of some patients with Burkitt's lymphoma (Manolov and Manolova, *Nature*, **237**, 33; 1972), and the number 13 interstitial deletion in retinoblastoma (Wilson, Towner and Fujimoto,

*Am. J. hum. Genet.*, **2**, 212; 1973). Not surprisingly, some tumours seem not to exhibit specific alterations in chromosomes, but show random changes (Popescu *et al.* *Int. J. Cancer*, **14**, 461; 1974) and others exhibit variability in malignancy correlated with the fluctuation in numbers of heterochromatic minute chromosomes (Shepard *et al.*, *Cytogenet. Cell Genet.*, **13**, 279; 1974). The interest of the material described by Codish and Paul lies in the apparently clear-cut nature of the phenomenon and one hopes that they will take advantage of the cell fusion approach to analyse it in more detail.

The tendency of certain tumours to exhibit predictable alterations in certain chromosomes, whether connected with increased malignancy or its suppression, presumably reflects a number of related factors such as the probability of the alteration occurring, the viability of the altered state and the predilection of certain carcinogens for causing damage at selected sites. It may be that certain chromosomes (for example those involved in nucleolus organisation) come more frequently into contact than others thus increasing the probability of interchange.

It seems quite certain however that particular groups of chromosomes are more frequently involved in formation of the marker chromosomes typical of a cell line than are others following virus transformation by adenovirus, SV40 and herpesvirus (Gallimore, cited in McDougall, *Prog. med. Virol.*, in the press). Chemical transformation of rat cells also results in the preferential involvement of three autosomes in marker formation (Olini and DiPaolo, *J. natn. Cancer Inst.*, **52**, 627; 1974) and two of these are regularly involved in the genesis of markers in adenovirus transformed cells (McDougall, *Prog. Med. Virol.*, in the press). An important future task will be to establish the mechanism of this effect. For this purpose virus-transformed cells seem ideal since they permit the use of specific probes which can detect integrated viral genomes, their transcriptional and translational products at the cell level. A beginning to such work has been made by the localisation of the control of SV40 T antigen to human chromosome 7 (Croce, Girardi and Koprowski, *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3617; 1973). Progress should be spectacular in view of the recent advances in analysis of the transforming genes of adenoviruses (see Rogers, *Nature*, **251**, 668; 1974).

K. W. JONES

## Arrival of the age of Rama

It is now generally accepted that the survival of the human race is threatened by twentieth century technology. Mankind must live with the uncertain hope that future innovation will not create more dangers than it removes, and occurs rapidly enough to forestall the collapse of organised industrial society.

Speculative science fiction enthusiasts have long dreamed of preserving humanity from earthbound destruction by colonising other planets. The stunning successes of near space exploration over the last decade have moved the discussion of extraterrestrial migration out of the domain of pure fantasy into the 'light relief' area of established science. But most of the colourful accounts published assume a depressingly high level of technology, in many cases decades or even centuries ahead of our present development. It is not now at all clear that technology will ever be able to reach the level at which exotic extraterrestrial solutions, such as the colonisation of Mars, will provide for the continuation of the human species. The global crisis has apparently arrived a few decades too soon.

If there is little ground for confidence in further gigantic technological leaps in the foreseeable future, it is heartening



to learn what radical possibilities for the extraterrestrial propagation of the human race are available with present day technological, economic, social and cultural constraints. One such possibility was put forward in *Nature* (250, 636; 1974) and received a detailed appraisal in *Physics Today* (27, 32; September 1974). In an article entitled "The Colonisation of Space", Gerard K. O'Neill, a professor of physics at Princeton University, outlines a scheme for constructing small "planetoids" in space for habitation by millions of people. The structure of these artificial worlds will be familiar to all those who have read Arthur C. Clarke's novel *Rendezvous with Rama*. They consist of a rotating cylinder, with the inhabitants living on the inside surface at normal gravity, in a completely self-contained ecosystem. The cylinders would be lit by sunlight reflecting from mirrors through glass or plastic cellular windows. Otherwise construction would be mainly from aluminium and titanium, with power requirements more than adequately met from solar radiation using conventional steam turbine electric generators.

Cylinders could be constructed in tandem, oppositely rotating and coupled so that they may always be orientated towards the Sun. A suitable place in space to locate them would be the  $L_4$  and  $L_5$  Lagrange libration points of the Earth-Sun-Moon system.

At first sight, Professor O'Neill's proposals appear to suffer from all the drawbacks of traditional space colony speculation; principally, the enormous energy expenditure necessary to transport a vast mass of material by rocket from the surface of the Earth. In fact, two crucial aspects of his ideas remove this objection. First, the project is self-perpetuating. With the establishment of a small scale pilot scheme providing an adequate environment for an industrial base, components for much larger cylinders could be manufactured and assembled *in situ*, thus reducing the problem to that of providing only raw materials. Second, the source of raw materials is suggested to be the Moon (and eventually the asteroid belt), the low gravity and vacuum conditions of which reduce enormously the energy expenditure required to transport ore to the colony. O'Neill discusses novel mechanisms, such as a two-arm rotor, the ends of which are moving at lunar escape velocity, which would propel pellets of lunar material at the rate of 3,250 tons per year, whilst operating at only 1,600 horse power. After a generation or two, the cylinder worlds would become entirely self-supporting.

In spite of the enormous possibilities available for this type of adventure using only current technology it is hard

to resist the impression that space Goliaths of this kind would not be economically viable. O'Neill has undertaken a cost analysis of the scheme, and claims that a kilometre-long, 10,000 population cylinder would cost about the same as the Apollo space project, thus being well within even national resources, and could be established as early as 1988. The cost of transporting 10,000 people to the colony could be met by the transportees themselves at, say, £10,000 per head. Larger cylinders would be able to pay for their own construction out of various space-based industrial products, such as high strength crystals.

Looking beyond the Solar System, there are available possible mechanisms for propagating humanity across the Galaxy. Although current rocket power can only achieve payload velocities of a few thousand miles per hour, travel time to nearby stars in the Galaxy is still only a few million years, that is, comparable with the age of the human race and a minute fraction of the life-time of most stars. Naturally, adult humans could not accompany a space capsule on such a venture, but millions of fertilised ova could easily be sent in a very small craft: The chief problems would be biological rather than mechanical. Craft such as these could then search the Galaxy for likely looking planets. One scheme might be to attempt to attract hypothetical extraterrestrial civilisations, which would then be given the responsibility of 'hatching' the nascent community. Alternatively this could be done automatically. The craft could be equipped with detailed biological and cultural information, so that the scheme would amount to a true extension of the human race rather than just putting flesh and blood on other worlds.

In the present social and economic climate, it is hard to imagine that schemes of this variety would get off the ground. The failure of space technology to provide any convincing major benefit to mankind has led to a rejection of large scale space exploration by the taxpayer. While space programmes were orientated towards 'glory trips' this was bound to happen. But against this background of muddled priorities projects such as O'Neill's are very appealing. Many direct benefits to mankind are apparent, not the least of which is a solution to overpopulation. Such projects could be undertaken as essentially a human, rather than a national venture, and provide a new perspective on the position of man in the Universe. They would also give a strong stimulus of confidence in a maligned technological system which, whether we like it or not, we can no longer do without. P. C. W. DAVIES

## A centenary in population biology...

from J. L. Harper

IN 1874 Carl Wilhelm Naegeli presented a paper to the Munich Academy of Science on "the displacement of plant forms by their competitors" (*Sitzb. Akad. Wiss. Munchen*, 11, 109; 1874). This represented perhaps the first attempt at a serious mathematical treatment of one of the fundamental questions in population biology and arose directly from Naegeli's reading of Darwin's *Origin of Species*. Publication of Naegeli's paper in the mathematics/physics papers of the academy was probably partly responsible for its failure to have any impact on the development of biological thinking. The paper is summarily mentioned by Gause (*The Struggle for Existence*; Williams and Wilkins, Baltimore, 1934) but otherwise seems to have been ignored in the development of a science that gained no significant momentum until 50 years later. Remarkably, this early essay in modelling of populations concentrated on problems in plant ecology whereas virtually all the subsequent development of experiment and theory in population biology has been concerned with animals.

Naegeli considered the consequences of introducing a new form into an already fully stocked population of plants. This contrasts with later developed theory which was usually concerned with simultaneous colonisation of an unoccupied habitat. He suggested that in a locality the average number of plants ( $z$ ) of any type reveals its relative strength against all other competitors. The average age of the individual plants ( $d$ ) and the annual replacement by young plants ( $e$ ) together determine ( $z$ ), both ( $d$ ) and ( $e$ ) depending on heredity and environmental influences. He recognised that when two species differ markedly in size, their numbers may be an inadequate description of relative abundance and the relative space occupied by the species might then be a better measure, but he avoided this problem by comparing pairs of closely related species. His ideal model was of a community of only two such species but "this very simple case . . . seldom occurs in nature". The seemingly more complicated but analogous case in which two closely related taxa live among vegetation composed entirely of other kinds of plants could be studied if the species ( $a$ ) and ( $b$ ) make the same or almost the same demands on other species in the community and compete in the same way as each other. In such a model ( $a$ ) and ( $b$ ) compete against each other but in a mutually exclusive way—sharing a total number of

individuals ( $a+b$ ) in proportion to their competitive vigour. (This treatment comes close to a formal definition of the 'niche'.) The question that Naegeli posed was: what changes occur in any given number of individuals, how do they succeed one another and what state of permanence do they achieve when certain assumptions are made about the length of life and conditions of replacement in both forms?

His model develops from  $z/d + z_1/d_1 = e + e_1$  where  $z/d$  and  $z_1/d_1$  are the annual wastages of the two forms (a) and (b) respectively and  $e$  and  $e_1$  the corresponding new colonists:  $e$  and  $e_1$  are variable but constant in their relation to one another. Naegeli used iterative procedures to show the simple consequences of varying parameters but historically the most fascinating part of the paper is his choice of special cases that he considered biologically relevant.

In the simplest model he assumed (1) that the life span of both forms and the ratio of their replacements depends solely on supposedly constant hereditary and ecological factors and that the values of  $d+d_1$  and  $e+e_1$  are independent of one another of  $z+z_1$ . He noted however that (2) the life span of a form may depend on its numbers or the numbers of its competitor (that is, density-dependent control of life span). He modelled the life span of a dependent on the numbers of  $a$ ; on the numbers of  $b$ ; and on the numbers of  $a+b$ .

He also modelled the three comparable situations (3) in which the annual replacement rate is dependent on the number of individuals. He envisaged positive effects such as the presence of older plants protecting seedlings and negative effects when the presence of many old individuals deprive young

growth of some limiting nutrient. Alternatively (4) the annual replacement of one form may be altered by the numbers of other forms or (5) the annual replacement of either form can be altered by the numbers of both forms acting together. He also considered interactions between the replacement rate and the life span: (6) the annual replacement can be modified by the life span of the individuals of the same form or (7) of the other form.

The essay uses the twentieth century concepts of density dependence and frequency dependence (though not the words). The models are deterministic but he was aware of the significance of chance events and noted the high chance of random extinction of a species when its frequency balances at very low numbers. Many of the interactions he chose to model were non-linear and he was particularly interested in models in which the square root of density was considered. He concluded that there were very many model situations in which even closely related species would not exclude one another. Today after an era in which most population biologists have struggled to explain diversity, it is curious to see the argument inverted with mutual exclusion regarded as the case needing explanation.

It was a remarkable vision which saw that the outcome of a struggle for existence depended on the comparative effects of intra- and inter-specific competition, that the effects might not be linearly related to density, that (though he did not develop the point further) it was practicable to define the effect of the density of one species by some factor into the effects of the density of another, and that the probability of extinction was relevant when numbers became small.

Naegeli had very great influence on botanical thinking in the nineteenth century. He made important studies of algal structure and life cycles and published classic early work on plant anatomy and ultrastructure. He contributed much to the debate on evolutionary theory and studied the mechanism of inheritance (unfortunately choosing the apomictic *Hieracium* as his experimental material). All of these activities are reported in the long obituaries by Vines (*Proc. R. Soc.*, **51**, 27; 1892) and Scott (*Nature*, **44**, 580; 1819) and in the outline of his life in Gillespie's *Dictionary of Scientific Biography* (9, Scribner, New York, 1974). None of these even mentions "The Displacement of Plant Forms by their Competitors". The paper must, in 1874, have seemed quite irrelevant to what biology was all about. In 1974 it has little to contribute to advancing ecological science but holds a fascinating place in the history of ideas.

## ... and a bicentenary for science writing

from Peter J. Smith

OLIVER GOLDSMITH, who died just 200 years ago, is one of those unfortunate writers whom most modern critics find it fashionable either to denigrate or ignore, but whose literary stature remains obstinately undiminished among a wider public. Admittedly, his masterpieces were few; Goldsmith's fame rests largely on one poem, *The Deserted Village*, one novel, *The Vicar of Wakefield*, and one play, *She Stoops to Conquer*. He was above all and by necessity a hack writer, though one who performed with skill.

By far the most important piece of hack work Goldsmith turned out was his *An History of the Earth, and Animated Nature* (1774), a remarkable eight-volume study. If it seems strange to find a poet, novelist and dramatist writing with some authority on a scientific subject, it needs only to be remembered that in the eighteenth century the disastrous split between science and the humanities had not developed to its present state. Moreover Goldsmith had studied medicine in Edinburgh and Leyden and would thus have had some feeling for the scientific methods of the day. As a writer to order he would in any case presumably have been willing to turn his hand to most subjects. Samuel Johnson is recorded as saying that if Goldsmith could distinguish between a cow and a horse that was about as far as his knowledge of animate nature went, although this flippancy was later to be balanced by the more serious admission that Goldsmith "has the art of compiling, and of saying everything he has to say in a pleasing manner. He is now writing a Natural History and will make it as entertaining as a Persian Tale".

So what was the nature of this compilation? The last seven of the eight volumes, which are concerned with the "animated nature" of the title, I leave to the naturalists to judge. Of particular interest to Earth scientists, however, is the first volume which is complete in itself. Goldsmith's purpose in this volume was to give a comprehensive view of the Earth and its physical, inanimate characteristics, which he does in the form of a progression from the globe as a whole ("a sketch of the universe") to more localised features such as mountains, rivers, tides and winds. On the way he deals with the Earth's surface, its internal structure and theories of its formation; he describes fossils, earthquakes and volcanoes; he speculates on the formation of new islands, the origin of rivers and erosion by the sea; he discourses

### Toehold for a toad





on air, meteors and the saltiness of the oceans. In short, he gives a general and accurate picture of how the Earth was viewed in the late eighteenth century; and he does so, moreover, within a logical framework which most writers of books about the Earth have adopted ever since.

As Goldsmith freely admits, the model for his first book was Buffon's *Histoire Naturelle*; but whereas Buffon was out to establish his theory of the Earth above all others and was quite happy to make some of the facts fit his case, Goldsmith had no axe to grind. He was concerned to be more critically objective and discriminating, and thus went beyond Buffon to Burnet, Woodward, Whiston and other sources, both acknowledged and unacknowledged. But from wherever his information came, his judgement was always on the alert and he was ever ready to discard the opinions of others, however eminent, if they were refuted by personal observation.

Goldsmith never lost sight of his intention to write not a scientific treatise but a popular account; his concern was to produce "innocent amusement for those who have nothing else to employ them, or who require a relaxation from labour". The result is a book which is both scientifically accurate (for the time), and a work of genuine literary merit written with what Jeffares (*Oliver Goldsmith*, British Council, 1959) calls "all the ease and grace we expect from Goldsmith". In seeking "to drag up the obscure and gloomy learning . . . to open inspection" rather than to publicise scientific advances or make a scientific case Goldsmith must be regarded as one of the earliest of what are now called 'science writers'.

During the following century, Goldsmith's *An History of the Earth, and Animated Nature* went into at least 23 editions, including one in Welsh. His model was copied by scores of writers during the nineteenth century. Could it be that Goldsmith's science writing and its legacy, ignored by scientific and literary historians alike, actually affected the public mind more than many of the better known purely literary works?

## A night on Mount Hamilton

by John Gribbin

THE Lick Observatory on Mount Hamilton, near San Jose, has an interesting history and a fine array of telescopes which make it well worth a visit from any itinerant astronomer (or ex-astronomer) who happens to be in the neighbourhood. I was in that happy position on October 25, and my native

guide, Dr John Faulkner, was also able to provide a potted history of the observatory. (For further details see the booklet *Lick Observatory*, fifteenth edition, available from the University of California, Santa Cruz.)

It seems that James Lick, who provided the finance for the building of the observatory, now rests in a tomb underneath the 36-inch refractor. He originally intended that the memorial by which the good citizens of San Francisco should remember him would be a pyramid downtown, rather larger than that of Cheops. He was dissuaded from this by a retired sea captain who pointed out the great lustre that could be brought to the name of Lick by an observatory containing the largest telescope in the world. After a costly site survey covering much of the United States (and the first of its kind) Lick and his seafaring friend were no doubt delighted to find that Mount Hamilton, in their own backyard, was as good a site as any available.

Building an observatory on the top of a mountain was an epic achievement in the 1870s. The builders did, in

fact, cheat a little on Lick's bequest, since they did not feel up to the task of building a reflector bigger than the Earl of Rosse's remarkable Irish instrument. Instead they built the largest refractor in the world.

Since those days, the Lick Observatory has expanded, and so, alas, has the nearby town of San Jose. The pretty lights below the mountain and the haze from the urban environment conspire to plague the astronomers. But although there is talk of a new observatory being built in a less accessible part of California, there are no definite plans as yet.

Pride of place on the mountain today is taken by the 120-inch telescope, which the Lick observers claim to be the best light bucket in the world. The impressive array of electronic gadgets which can be attached to it makes the telescope, they claim, a better performer even than the 200-inch at Palomar, although the Lick instrument has just 36% of the collecting area of the larger telescope. But they may soon have to consider a new rival to the telescope which can get the most in-

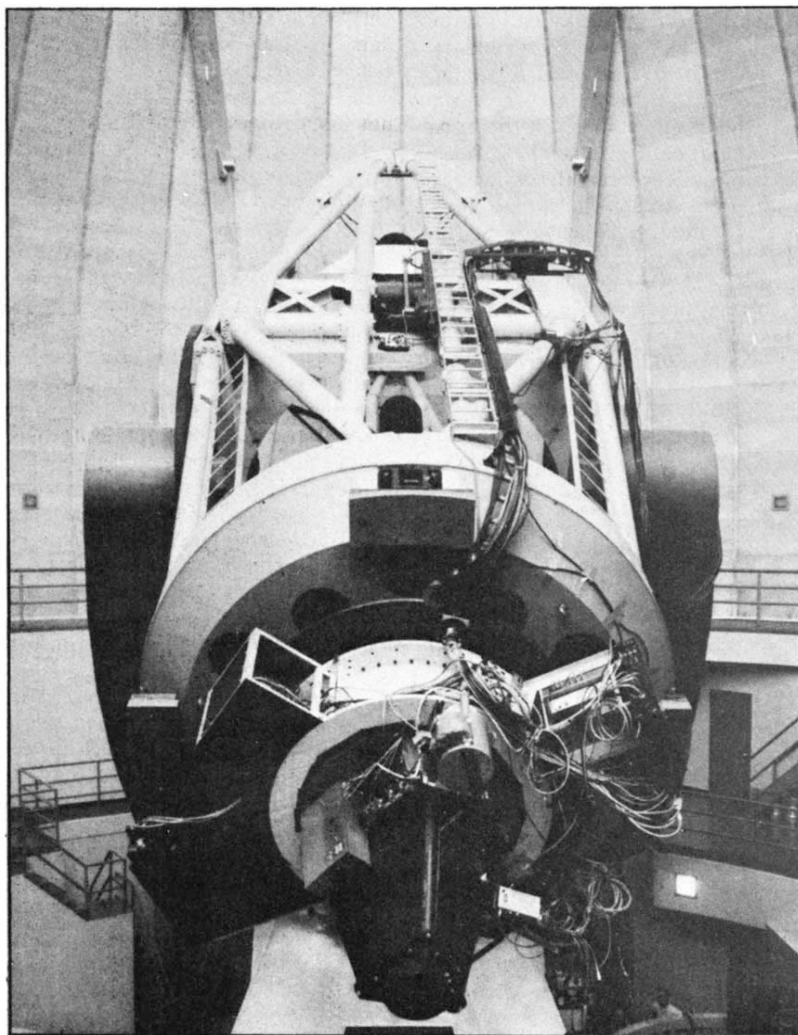
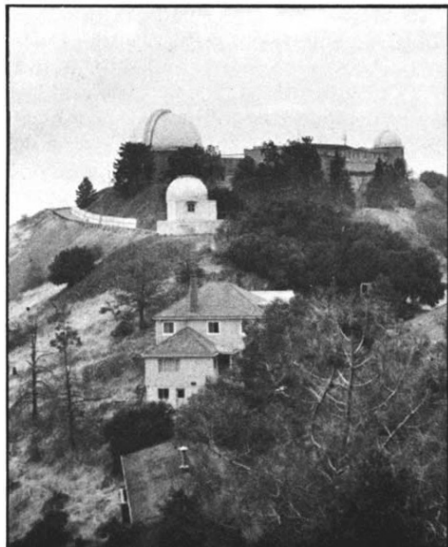


Fig. 1 The business end of the 120-inch telescope on Mount Hamilton, showing the impressive array of electronic instrumentation. (Courtesy Lick Observatory.)



**Fig. 2** "It was a dark and stormy night . . ." View of the main building of the Lick Observatory from close to the 120-inch site. The small hut in the middle ground conceals the absence of any electronic instrumentation on the Tauchmann.

formation out of the smallest number of photons—the Anglo-Australian Telescope, whose director, Jo Wampler, must have taken with him from California many of the tricks of the trade.

But the casual passer-by is not allowed even to enter the 120-inch dome, much less operate the mighty machine. For such itinerants, a slightly less sophisticated instrument with an aperture one sixth that of the big telescope is available. This instrument, the Tauchmann reflector, provided the entertainment on the night of my visit. In the words of one recent user it provides "a rickety but fun operation". The meaning of this can best be gauged from the instruction book provided to aid the unfamiliar with their observations. After explaining how to open the dome (not as easy as the uninitiated might guess) and how to set the telescope in the required direction, some handy hints are provided to explain why you may not be able to see anything. The list begins: "(1) Is telescope pointing out of dome slit?" and continues in similar vein.

Our fun was only slightly marred by the 99% cloud cover on the night of October 25. We did, briefly, see the Moon and my colleague claims that he saw Jupiter, but the observation was not confirmed. As the only observers on the mountain that night we can at least report that for once the Tauchmann achieved more than the 120-inch. Of course after a two hour drive back to Santa Cruz on the coast we found a cloudless sky, thus proving Parkinson's second theorem.

## Chunky sputtering

from Robert W. Cahn

THOSE incorrigible optimists who look for salvation to the large-scale controlled release of thermonuclear fusion energy should be cheered by a conference which was held at Argonne National Laboratory near Chicago last January, under the title *Surface Effects in Controlled Thermonuclear Fusion Devices and Reactors*. The proceedings have now been published as a special volume of the *Journal of Nuclear Materials*. For perhaps the first time, a major conference took as a premise that large fusion reactors will be built well before the century is out; Klaus Zwilsky of the Division of Controlled Thermonuclear Research of the United States Atomic Energy Commission proffered a timetable which included an experimental power reactor in operation by 1984. The conference was concerned with one class of engineering problems which will arise in the design of such reactors, namely the interaction between imperfectly confined plasmas and the wall of the containing vessel, combined with more conventional radiation damage due to energetic particles, especially neutrons, impinging on the walls. Physicists and metallurgists do not lavish time and money on such difficult studies unless hard-headed engineers have concluded that the hardware is in principle feasible.

Many of the papers dealt with the effects of particles peculiar to thermonuclear reactors, such as lithium, deuterium and tritium ions, but a particularly intriguing group of papers was concerned with that familiar entity, the high-energy neutron. The neutrons impinging on a fusion reactor wall will be exceptionally energetic, and little is known concerning the effects of such super-neutrons. Kaminsky and Das (*J. nucl. Mater.*, **53**, 162; 1974) experimented with 14 MeV neutrons from an accelerator, impinging on niobium. It has long been realised that the sputtering (that is, collision-induced erosion) of the metal could gradually damage the wall, and perhaps more serious, contaminate the plasma with metal ions through the knocking-out of individual metal atoms. But something else quite unexpected happened as well. 'Chunks' of niobium often exceeding one micrometre in size and containing many millions of atoms, were emitted from the surface. Nothing remotely like this had been reported before in sputtering experiments. Kaminsky and Das discovered that the emission of chunks was much greater when the target was cold-rolled and roughly polished than when it was annealed and finely polished; though

these aspects were not varied independently, the investigators were sure that internal stresses and rough surfaces each and severally promote emission of chunks (as opposed to single atoms).

A companion paper by Guinan (*ibid.*, page 171) develops the hypothesis that thermal transients engendered by collision cascades within the metal will generate shock waves which might suffice to spall off chunks. Guinan concludes that the magnitude and duration of such shock waves would not alone suffice to do the trick. But if the metal contains regions of high internal stress (for instance, at the head of a dislocation pile-up) then as Guinan shows, a shock wave can act as a trigger to release the stored internal energy, leading to the ejection of a chunk. On this hypothesis, as Karminsky and Das have found, both plastic deformation and surface roughness will foster chunk emission (though it must be admitted that the role of the second variable is not clearly explained). Unannealed cold-rolled foils, which contain macro as well as micro stresses, are particularly vulnerable. Karminsky and Das conclude that a high mirror polish on a vacuum-annealed metal, monocrystalline for choice, will render it virtually immune to chunk emission or the associated damage. It seems the phenomena resembles ordinary fatigue, very largely controlled by surface quality.

In related studies, Blewitt *et al.* (*ibid.*, page 189) failed to find chunks emitted from gold bombarded with 14 MeV neutrons, whereas Biersack, Fink and Mertens (*ibid.*, page 194) found copious chunk emission when  $\text{UO}_2$  films were sputtered by self-generated fission fragments during neutron irradiation. Here there was a pronounced effect of foil thickness (thin foils emit more chunks), and in particular, there was a lower limit of neutron dose below which no chunks emerged. It is surprising that the many earlier experiments on fission-fragment self-sputtering had not previously uncovered this phenomenon.

The notion, which seems well supported by the experiments on niobium, of the cold-rolled metal as a sort of self-stressed microbomb from which fragments are released by neutron bombardment, is reminiscent of researches at Harwell in 1956 by Cottrell and Roberts, who discovered that the intergranular stresses engendered by uranium by the anisotropic radiation-induced growth of individual crystal grains led to creep under absurdly small applied stresses. Here, the external stress triggers off the release of internal stresses created by prolonged uranium fission. Radiation creep has come to be recognised as a



major hazard in fission reactions, and it may be that chunk emission must be guarded against with equal care in the fusion reactors of the future.

## Cancer in Tuscany

from R. A. Knight and N. A. Mitchison

BANNERS decked the streets of Florence, Sienna, Montecatini, Pisa and Lucca, for the 6,000 members who came to listen to papers given at the Eleventh International Cancer Congress by some 4,500 authors on October 20–26. Buses and trains shuffled to and fro between the five cities, but we found the delight of autumn in Tuscany compensated for the fuss. The congress accurately conveyed the flavour of cancer research over the past four years: greatly to oversimplify it was one of slow progress in cell biology and immunology, with dramatic news concerning viruses. We listened to the virology and immunology papers.

In the RNA tumour virus field, new work localised specific viral antigens on the tumour cell surface. D. P. Bolognesi (Duke University) showed that monospecific anti-p30 and anti-gp71 sera were cytotoxic for virus producer and non-producer methylcholanthrene induced mouse tumours, although the anti-p30 effect may be mediated by antigen non-specifically absorbed to the cell surface. Natural mouse antibody is also cytotoxic for these cells, and can be partially absorbed both by gp71 and by p30. The predominant anti-viral antibodies in the serum of normal wild and laboratory strain mice, reported by R. C. Nowinski (University of Wisconsin), react with p15, and with both viral envelope glycoproteins. Nowinski also finally laid to rest the old dogma that mice cannot mount a humoral anti-p30 response, since this is the major specificity in the serum of C57BL mice immunised with the K36 leukaemia.

Several workers discussed the relationship of oncogenic viruses to human tumours. G. Klein (Karolinska Institute) reported that although 98% of African Burkitt lymphoma biopsies were positive both by nucleic acid hybridisation for EBV DNA, and for the Epstein-Barr nuclear antigen, European cases were negative for the genome. S. Spiegelman (Columbia University) summarised evidence from his laboratory for an association between 70S RNA and a reverse transcriptase-containing particle in a variety of human tumours, and showed that this agent contained a protein which cross reacts with p25 from the Mason-Pfizer agent. R. C. Gallo (National Institutes of Health) found that antisera to primate virus reverse transcriptase cross react with an antigen from human leukaemic cells,

and that human tumour cells contain an antigen cross-reactive with primate virus p30. Visual sightings of the mature C-type particles in human tissue material, however, are rare. Non-enveloped particles, which contain 70S RNA and viral antigens (virosomes), and which actively incorporate labelled precursors *in vitro*, were described in the mitochondrial membrane of avian and murine tumours by J. Kara (Czechoslovak Academy of Sciences, Prague). These particles are more prominent in non-producer tumours. Although the Spiegelman particles, but not the oncornavirus precursors identified in human cell lines by A. Bukrinskaya (Academy of Medical Sciences, Moscow), differ from virosomes in density, more than one worker went home anxious to isolate human mitochondria. Thus immunology's most useful contribution to cancer research at present may be in the identification of candidate human tumour viruses.

Many papers dealt with the immune response to tumour-specific antigens. On the clinical side, the meaning of cell mediated immunity *in vitro* was questioned in several papers, for example by M. Takasugi (University of California, Los Angeles), R. W. Baldwin (University of Nottingham), and F. J. Cummings (Brown University): the popular microcytotoxicity test can no longer be accepted as a measure of the response of the patient to his own tumour. Significant responses, if they occur at all, seem to do so most clearly with a restricted range of tumours which include bladder carcinoma, melanoma and sarcomas. Encouraging results were reported with 'immunotherapy'—both specific (with administration of tumour cells or membrane preparations) and nonspecific (with immunological adjuvants such as tubercle bacilli, where C. Maruyama (University of Tokyo) had particularly extensive data). In no case, however, was there decisive evidence of the involvement of an immune mechanism.

The use of immunological methods to detect and monitor tumour growth was concentrated mainly on carcino-embryonic antigen for carcinoma of the gut and alpha-fetoprotein (AFP) for hepatoma, with limited use of cellular immunity against cell lines in other types of cancer. Papers from Europe and the United States, all of which took a cautious line towards widespread population screening were dwarfed by one from the Coordinating Group for AFP (Pekin) which included data from a massive screen of 500,000 individuals. Those who wish to evaluate the test wait, therefore, to see whether the Chinese find the effort worthwhile.

Analysis of the immunological mechanisms which operate in animal tumour systems continues. The entire response

from induction to final killing can now be obtained *in vitro*, and the killer cells thus generated are highly active (J. C. Cerottini, Institute of Cancer Research, Lausanne). Efforts to characterise the antigens involved have now reached the stage reached 5–10 years ago with the major transplantation antigens. Virus-coded cell surface antigens are better characterised, and are available in some instances for *in vitro* manipulation with lymphocytes (G. R. Shellam, Imperial Cancer Research Fund, London). From the strictly immunological standpoint the furthest advance was reported by K. Rajewski (University of Koln), who has rendered antigens obsolete by eliciting a response with anti-idiotypic antibody, that is, with antibody directed against the antigen-combining region on the receptors of a clone of lymphocytes. Since his procedure stimulates T lymphocytes as well as B lymphocytes, it implies—subject to certain safeguards in interpretation—that T and B cells read from a common dictionary and share a common pool of receptors.

## Tumour viruses at Munich

from a Correspondent

TUMOUR virus meetings tend to have the same speakers, discuss the same subjects and send their participants away asking the same question: what do tumour viruses have to do with tumours? The clinical oncologist must helplessly face this question every day; meanwhile the basic oncologist faces the statistics that 60–90% of all known malignancies in man are caused by chemical carcinogens and that in no case so far have the candidate human cancer viruses been firmly identified as a cause of cancer in man. Their problems are intensified by the gap between experimental tumour research and clinical oncology. The tumour virus meeting held at the Max-Planck Institute for Biochemistry, Munich, from October 4 to 10 provided a good opportunity for communication between basic and clinical oncologists.

Dr R. Gallo (National Cancer Institute, Bethesda) discussed his concept of class I and class II viruses. He showed that the RNA genome of class I viruses (typical endogenous viruses such RD-114) hybridises virtually completely to DNA of normal uninfected cells. These virogenes may be incompletely expressed and their products, such as reverse transcriptase, can be associated with normal tissues. Class II viruses show much less hybridisation of their RNA genome to uninfected cell DNA. Members of this class, for example murine and feline leukaemia

and sarcoma viruses, can probably be transmitted horizontally.

Dr S. Spiegelman (Columbia University) who has little faith in inherited oncogenes, presented evidence which certainly speaks in favour of horizontal transmission. In his studies with identical twins he has found that the leukaemic twin contained particle-related DNA sequences that could not be detected in the leukocytes of his identical healthy sibling. Dr S. Dube (Max-Planck Institute, Göttingen) suggested that the mechanism of neoplasia could involve somatic hypermutation of vertically transmitted endogenous type C virogenes; if this hypothesis is correct, Dr Spiegelman would have seen no difference between leukaemic and healthy siblings if he had looked at germ line rather than leukocyte DNA.

Work on viral structural proteins is progressing rapidly. Drs Moenning and Hunsmann (Max-Planck Institute, Tübingen) described the purification and characterisation of the major coat protein p30 (molecular weight 30,000) and the major envelope glycoprotein gp71 of Friend erythroleukaemia virus. They showed that gp71 must reside in the surface knobs because treatment with bromelain removes both knobs and gp71. Dr M. Strand (Albert Einstein College, New York) reported that she finds an additional glycoprotein, gp69. Each of these proteins contains distinct interspecies antigenic determinants. The gp69/71 mixture can induce neutralising antibodies and thus seems to be potentially the most useful antigen for the preparation of vaccines. Dr F. Lilly (Albert Einstein College, New York) showed that gp69/71 is non-coordinately expressed with the p30 protein but appears to be partially linked to sarcoma genes. Dr Vaheri (University of Helsinki) using radioimmunoassay, showed that leukosis-free chicken cells expressed low concentrations of p30, but after infection the cellular concentration of p30 went up by 100–1,000 fold.

The nature of the viral genome was actively discussed. It is generally agreed that the genomic RNA is a 70S aggregate (molecular weight  $10^7$ ) of several 30–35S (molecular weight  $\sim 3 \times 10^6$ ) subunits. Whether the subunits are identical or not has been a major issue. From the extensive genetic and biochemical evidence accumulated by Dr Vogt (University of Southern California, Los Angeles) and Dr Duesberg (University of California, Berkeley) it seems that the genome of Raus sarcoma virus is polyploid; that is, the subunits are identical. This is supported by the independent findings of Drs Baluda (UCLA), Billeter (University of Zurich) and Dube.

Drs Wu and Gallo have been

studying compounds which affect virus replication and virus-induced transformation and which may therefore have clinical value. Dr Wu described the inhibitory effects of rifamycin SV derivatives on proviral formation and cordycepin on virus production at an early post-transcriptional step. Dr Chandra (Frankfurt) reported on the inhibitory effect of thio-poly-nucleotide homopolymers of cytidylic acid and uridylic acid.

## Energy in agriculture

from N. R. McFarlane

At a meeting on October 21 held at the Society of Chemical Industry, chemical and mechanical aspects of energy utilisation in agriculture were discussed.

J. J. Landsberg of Long Ashton Research Station pointed out that the conversion of solar energy into useful biomass is an inefficient process. Man's efforts to increase useful biomass in agriculture moreover require considerable energy input in the form of fertilisers (Professor G. W. Cooke of Rothamsted Experimental Station). While other agricultural chemicals such as herbicides are less energetically demanding, requiring similar energy inputs to mechanical weeding methods (Dr M. B. Green of Imperial Chemical Industries) they are also less effective than fertilisers. The link between solar sources of energy and chemical energy was provided in the discussion of the direct use of coal, oil and gas for heating and mechanical operations on the farm and in the glasshouse (G. F. Sheard, Glasshouse Crops Research Institute).

The paper which created most discussion was that of Dr K. L. Blaxter of the Rowett Research Institute, who showed that known world reserves of energy ( $42 \times 10^{15}$  MJ) are equivalent to but two years of European sunshine! His Table 1 shows that only 11% of the total plant material harvested in the United Kingdom is 'farm gate' output, destined for human consumption. After processing this primary plant food

**Table 1** Biological energetics of agriculture in the United Kingdom

|                                   | Energy<br>(MJ $\times 10^9$ yr $^{-1}$ ) |
|-----------------------------------|------------------------------------------|
| Solar radiation total incidence   | 610,000                                  |
| Gross primary production          |                                          |
| harvested from plants             | 1,116                                    |
| Imports of foodstuffs             | 104                                      |
| 'Farm gate' output of             |                                          |
| crops                             | 119                                      |
| 'Farm gate' output of             |                                          |
| animals                           | 94                                       |
| Total farm output                 | 213                                      |
| Edible food from crops            | 65                                       |
| Edible food from animals          | 73                                       |
| Total edible food                 | 137                                      |
| Energy required by the population | 241                                      |

**Table 2** Energy inputs for agriculture in the United Kingdom

|                          | Energy<br>(MJ $\times 10^9$ yr $^{-1}$ ) |
|--------------------------|------------------------------------------|
| Fuel                     | 144.5                                    |
| Fertilisers and lime     | 128.1                                    |
| Agrochemicals            | 1.2                                      |
| Machinery                | 48.8                                     |
| Processing of foodstuffs | 2.1                                      |
| Transport                | 15.7                                     |
| Total                    | 340.4                                    |

yields  $65 \text{ MJ} \times 10^9 \text{ yr}^{-1}$  of energy for human consumption. 70% of the prime plant output is eaten by farm livestock—a reflection of the importance of the animal sector in British agriculture. Table 2 was also presented by Dr Blaxter to show the high energy inputs besides solar energy needed to maintain the agricultural output.

Both Professor E. W. Russell (University of Reading) and Dr R. J. Martin of Plant Protection Limited criticised short term economical evaluation of depleting natural resources. The message I received was that true economic value will not be placed upon such items as fossil fuels until it is too late. Even the use of conventional economic evaluation under such circumstances is somewhat suspect.

## Immunity and connective tissue

from A. C. Allison

THE Fifth Lepetit Colloquium on The Immunological Basis of Connective Tissue Disorders was held in Madrid on November 11–13. M. Mannik (University of Washington) described the self-association of IgG rheumatoid factors to form stable dimers in which one antibody binding site on each molecule reacts with an antigenic determinant on the Fc region of the other molecule. The calculated association constant for this dimerisation is remarkably high ( $10^{10} \text{ l mol}^{-1}$ ). In addition a concentration-dependent aggregation of dimers into tetramers, octamers and higher polymers is observed. The self association of rheumatoid factors may play a part in perpetuating immunologically mediated synovitis. Complexes of IgG and IgM antiglobulins that precipitate in the cold (cryoglobulins) are sometimes associated with renal disease (E. Franklin, New York University) and some patients with systemic lupus erythematosus (SLE) have been found to have idiopathic markers of monoclonal cryoglobulins in their renal lesions (H. Kunkel, Rockefeller University).

Several laboratories have been studying the antibodies against human T and B lymphocytes, some reactive only in



the cold and others at 37° C, present in patients with SLE, rheumatoid arthritis (RA) and infectious mononucleosis. Messner (Albuquerque) reported the presence of cytotoxic antibodies against lymphocytes in the majority of family members of patients with SLE, irrespective of consanguinity. A virus or other transmissible agent may be involved.

F. Dixon (Scripps Clinic, La Jolla) emphasised the analogy between the role of endogenous virus antigens in mice and the appearance of SLE in humans. In all mouse strains virus-specific antigens are found, and in some strains complexes of these antigens and antibodies, as well as nucleic acids and antibodies, accumulate in the renal glomeruli.

R. Lerner (Scripps Clinic, La Jolla) presented evidence obtained in his

laboratory and that of E. Boyse in New York that a mouse thymocyte differentiation marker,  $G_{IX}$ , is the same as a glycoprotein (gp70) in the virions of murine oncornaviruses. The oncornavirus genomes are integrated in the host genomes, and the expression of gp70 is under control of factors both genetic (manifestation on the membrane of thymocytes in  $G_{IX}^+$  strains only) and environmental (manifestation only under the influence of the thymus). In  $G_{IX}^-$  strains the antigen is present only in leukaemic cells. Thus the distinction between virus and host gene products becomes tenuous, and whether immune responses to them are regarded as autoimmune is a question of semantics. As in mice and chickens, all human cells seem to have in their genomes lengths of DNA hybridising with oncornavirus nucleic acid, and

the number of copies of oncornavirus DNA is increased in SLE and leukaemia.

B. Pernis (Basel Institute for Immunology) pointed out that most human B lymphocytes have surface IgM as well as IgD, the proportion varying from cell to cell. These turn over at different rates: when they are lost after capping or protease treatment IgM is resynthesised much more rapidly than IgD. W. Hijmans (Institute for Experimental Gerontology, Rijswijk) reported that the only surface immunoglobulin of lymphocytes in young human foetuses is IgM, which invalidates the view that IgD is the first to appear.

H. Muller-Eberhard (Scripps Clinic, La Jolla) described the amino-acid sequence of the complement component C3. The cleavage product C3a requires a high  $\alpha$ -helical structure for activity. When C-terminal arginine is removed from C3a, the product binds to mast cell receptors and norepinephrine receptors of blood vessels and blocks their activation. K. Austen (Harvard) emphasised the symmetry of the steps involved in activation of complement by the classical and alternate pathways. Both pathways are activated in the joint fluids of rheumatoid arthritic patients. Austen concludes that the alternative pathway is activated *ab initio* but another explanation, that there is feedback activation from the classical pathway, is difficult to exclude.

J. Natvig (Institute of Immunology and Rheumatology, Oslo) reviewed recent observations on the heterogeneity of amyloid. A non-immunoglobulin AA protein from tissues of different patients shows identity over most of the 76 residues but some variation in the C-terminal region. Levels of the related serum component (SAA) are increased in sera of patients with rheumatoid arthritis and in old people. An immunoglobulin isolated from amyloid shows homology with  $\lambda$ -chains over the first 45 residues and seems to represent a new  $\lambda$ -variable subgroup.

L. Glynn (Kennedy Institute, London) discussed his model of persistent monoarticular arthritis following intra-articular injection of antigen into previously immunised animals. Complexes of antigen and antibody are found in menisci, but it is not clear whether these have any pathogenetic role. Release of hydrolytic enzymes from mononuclear phagocytes exposed to immune complexes or products of activated lymphocytes, discussed by A. Allison (Clinical Research Centre, Harrow), may be involved in joint damage in rheumatoid arthritis. There is still no convincing evidence of any causal role of an infectious agent in rheumatoid arthritis.

## Immunopathology of parasitic infection

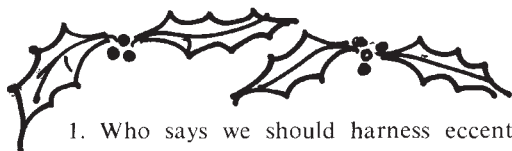
F.E.G.C. REPLIES to the responses in Matters Arising last week (252, 509; 1974) to his News and Views article (246, 187; 1973).

My statement that "most parasites are capable of evoking immune responses in their hosts but these are seldom effective in eliminating the infection" implies that some parasites do mount effective immune responses. Professor Urquhart and Dr Miller both refer to lungworm disease of cattle thus stressing the comparative rarity of the latter situation. It is true that there are well defined immune responses that eventually result in some degree of protective immunity against parasitic infections. But it is equally true that the actual elimination of the parasites as a result of an immunological response is not commonplace. The major parasitic diseases of man (malaria, sleeping sickness, Chagas' disease, American leishmaniasis, schistosomiasis and filariasis) are characterised by infections that last months or years. To most people this indicates a failure of the immune response to eliminate the infection. The immune response may ameliorate the disease symptoms but transmission of the parasite still occurs. Domesticated animals are more fortunate than man in being able to mount effective immune responses but in piroplasmosis and fascioliasis these responses are not very effective. It must also be remembered that helminth infections may diminish as a result of the natural death of the worms and not an immune response. This aspect of recovery from infection has been

little studied.

My statement that "the possibilities of developing useful methods of immunisation [against parasitic diseases] fade further and further into the distance" does not imply that immunisation is always going to be impossible. Vaccination against cattle lungworm, canine hookworm and certain kinds of piroplasmosis has been achieved but the vaccines used, irradiated larvae or infected blood, are unacceptable in the field of human medicine. With the development of a vaccine against cattle lungworm nearly twenty years ago, it was felt by many workers that the solution to the problem of vaccination against parasitic infections was just round the corner. This early optimism has not been justified. As more and more is discovered about immunity to parasitic infections further complications are uncovered. The development of vaccines against many parasitic diseases may not be possible before the diseases are eliminated by drugs or public health measures. Trichinellosis is a case in point. There have been encouraging reports of possible immunisation procedures against *Trichinella* but its decline in the United States has been brought about by legislation designed to prevent swine fever. Studies on the immunological responses to parasites have been amply rewarded by discoveries in pure immunology, the development of immunodiagnostic techniques and an understanding of many immunopathological conditions. Vaccines will also be developed as a result of these studies—but not for many years to come.

# Christmas quiz



1. Who says we should harness eccentric buoys?
2. What is the next number in the series 1, 2, 3, 5, 8, 13, 21, 34, 55 ... and in what way is this series linked both to Pisa and to leaves?
3. What professor of chemistry wrote an opera whose most famous tune was, many years later, used in the musical 'Kismet'?
4. She wanted his next book dedicated to her and indeed it was—but instead of being a story of wide popular acclaim, it was an elementary treatise on determinants. Who was she?
5. Who has an answer to Logical Positivism in the 'family Four Position Base'?
6. Appearing twice a year, they are common in India, China and Japan but are probably indigenous to western Asia. In ancient Egypt one variety was accorded some degree of divinity and is featured on monuments of that period. If you had one what would you do with it?
7. He studied in Vienna and by the age of 26 had become Professor of Mathematics at Gratz University. Four years later he moved to the Chair of Physics in Vienna, and it is as a physicist and psychologist that he is remembered most. Towards the end of his life he became a member of the Austrian house of Peers. The rather one sided view from one of his philosophical works was featured briefly in a recent edition of *Nature*; what was it and who was he?
8. What was the concept that was first suggested publicly in an address in Frankfurt am Main in January 1912; and who delivered the address?
9. Where and when did the largest single oil spill occur?
10. What is the most commonly used tricyclic antidepressant and what are the main effects of such compounds?
11. What is dendrochronology and what importance does it have in art?
12. Of what biochemicals are these the empirical

formulae: (a)  $C_{35}H_{28}MgN_4O_5$ ; (b)  $C_{63}H_{88}CoN_{14}O_{14}P$ ; (c)  $C_{31}H_{32}ClFeN_4O_4$ ?

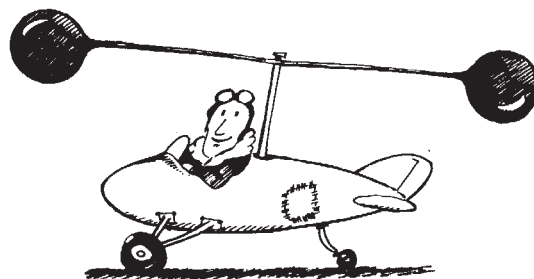
13. Name the group which in 1974 officially wanted restriction restricted?
14. By what discovery in 1974 was St Bartholomew's Hospital specifically linked to a gene deletion?
15. Which enzymes are deficient in: (a) Fabry's disease; (b) Sandhoff's disease; (c) Pompe's disease?
16. Who saw fruit in a padded cell?
17. From where, precisely, was Marconi's first trans-atlantic radio transmission made?
18. How many optical telescopes bigger than 100 inches are in operation or under construction in the Southern Hemisphere and where are they?
19. Some useful quotations for authors to know—but who said them first?
  - (a) Accuse not Nature, she hath done her part ...
  - (b) can't be Nature ... not sense ...
  - (c) except the blind forces of Nature, nothing moves in this world ...
  - (d) The fault was Nature's fault not thine ...
  - (e) What's a' the jargon of your schools;  
Your Latin names for horns and stools;  
If honest Nature made you fools.
  - (f) Thrice happy he who not mistook  
Hath read in Nature's mystic book ...
  - (g) to be constant, in Nature were inconstancy ...
  - (h) Nature is always wise in every part ...
  - (i) Nature abhors imperfect work ...
  - (j) But in them Nature's copy not eterne ...
  - (k) Nature knows a thing or two ...
20. Who wrote:  
Nature and Nature's laws lay hid in night:  
God said let Newton be! and all was light  
and who replied with what?
21. For what work did the following win Nobel Prizes:  
D. Bovet (1957); J. Heyrovsky (1959); G. von Békésy (1961); G. Wilkinson (1973)?  
See page 622 for answers.

## Spinning into space?

RECENT publicity about possible 'antigravity' devices which seem to defy Newton with the aid of a spinning top or two encourages us to offer for seasonable speculation the following question, taken from the 1969 Christmas Examination for Junior and Senior Honours in the Natural Philosophy Department of the University of Aberdeen. There is no prize for any correct answer, and no answer is presented in the pages of this issue of *Nature*.

"There is currently an application for a patent for an antigravity device based on the following argument:

'A particle at the Earth's surface has a critical horizontal velocity  $v$  such that this velocity will cause it to orbit the Earth in a circle. If this velocity is exceeded, the particle will tend into an orbit at a greater mean distance from the Earth's centre. The actual direction of the velocity in a horizontal plane makes no difference to the foregoing conclusion. Now such a horizontal velocity can in principle be achieved by spinning two particles joined by a horizontal bar as in a dumbbell. Each particle can thus be



made to have a velocity  $V$  which is greater than  $v$ , and so they should jointly rise. The proposed method of defying gravity therefore consists in spinning a dumbbell fast enough.'

What is the flaw in the argument, and how would you try to persuade the inventor (an imaginative engineer who might not be convinced by an argument based on the motion of a common centre of gravity) that he would be foolish to waste money on pursuing the patent?"



# Science journalists come of age in Pisces

John Gribbin\*

*It seems that more science journalists are born under Pisces than any other sign.*

WINDSOR<sup>1</sup> has pointed out that the astrological belief in the importance of the 'Sun sign' as an influence on personality seems to be reflected in the distribution of birth dates of American biologists. In particular, he found that molecular biologists tend to be born under Aries. I have now compared the birthdays of science journalists found in the London office of *Nature* with those of all staff members found in that office. The birthdays were obtained by personal questioning during August, 1974, and the appropriate Sun signs were assigned.

More science journalists were born under the sign of Pisces than any other sign (Fig. 1), and relatively few (zero) members of the *Nature* staff were born under Aries, Taurus or Gemini. In spite of the small size of the sample, the result is significant as indicated by Lynden-Bell's half-power test. This test uses the criterion that in statistics, all numbers go as the square root (D. Lynden-Bell, unpublished).

Taking the data for science journalists alone, there are eight Pisces and eight others (including one "no comment"). The most densely populated Sun sign other than Pisces is Leo, with just two members—less than even the half-power of the Pisces population.

It may also be significant that of the Leo population one (E) is the Editor of *Nature* and the other (DE) is Deputy Editor.

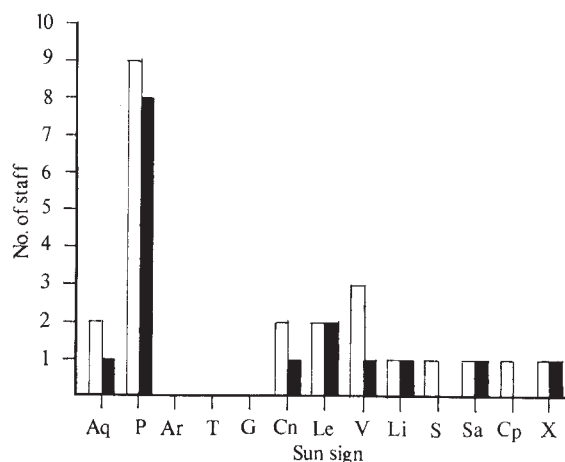


Fig. 1 Frequencies of Sun signs of *Nature* staff. Open histogram, all staff; solid histogram, science journalists only; X, birthday not revealed ("no comment").

While these results are suggestive, further observations of a larger sample are needed before any firm conclusions can be drawn.

<sup>1</sup> Windsor, D. A., *Nature*, **248**, 788 (1974).

\* *Nature*, London

## Ambisonic reproduction of directionality in surround-sound systems

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*In both the technology and the aesthetics of extending high fidelity reproduction to surround-sound, reproduction of natural ambience is crucial. The 'quadraphonic' attempt to reproduce four stereo-blended tracks, derived from multi-microphone mix-down, cannot provide this. Complete spherical directionality can however be encoded on to a minimum of two audio channels to produce acoustically acceptable surround-sound systems. Limitations are set both by the number of available loudspeakers and by the number of channels.*

THE history of representational art has many examples of what people of a particular age and culture were unable to perceive, and therefore to reproduce; for instance the inability of the dynastic Egyptians to perceive that the full-face eye is not seen in a profile figure. The reproduction of sound follows a similar pattern. In the early development of the phonograph and of 'wireless' the listener was so satisfied with the novelty of hearing the human voice or music issue from a machine that he demanded nothing else. Indeed there was often little conscious

appreciation that anything was lacking in fidelity of reproduction; how else could Sir Arthur Conan Doyle suppose in *The Adventure of the Mazarin Stone* that Sherlock Holmes could deceive a sophisticated villain into mistaking a 'modern gramophone' of 1927 for real violin playing, even in the next room. As late as the 1930s, many listeners could see little short of perfection in the commercial 'radiogram'.

As the sense of wonder wore off, however, a more critical attitude filtered down from audio engineers to the buying public, and the search began for high fidelity reproduction.

During and immediately after the Second World War, domestic reproduction of sound depended on the 78-r.p.m.

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shellac disk and a.m. broadcasting. Now with the vinyl disk and f.m. broadcasting, high fidelity reproduction has reached the stage at which the best equipment can make a chamber group sound (to me) like the real thing; but this degree of realism in the reproduction of an orchestra still eludes us. Careful introspection suggests that even this judgement is too lenient, and that we are 'listening-through' major defects which have always been inseparable from reproduced sound, and which therefore we take for granted.

One aspect that is obviously lacking is directionality. Monophonic reproduction gave no explicit directional information, even when reproduced from more than one loudspeaker. In stereophonic reproduction, sound reaches the listener only from the forward direction, whereas in the concert hall the listener is bathed in reverberant sound coming from all directions. In addition, stereo works by feeding common signal-components to a pair of loudspeakers with relative amplitude determined by the position which the illusory sound source is to occupy. It is easy to show that the perception of stereo-imagery is both physically artificial and aesthetically untrue to life; it has to be learnt and some 10% of the population are unable to do this. Moreover, although skilful recording can give some illusion of depth, the acoustic images are essentially strung out along a line joining the two loudspeakers, giving an effect that has been described as a cardboard cut-out orchestra.

## Role of directionality

From the antiphonal tradition through to the school of St Mark's and the baroque, directionality played a large and explicit part in western music. In classical and romantic orchestral music this explicit component is largely abandoned, some composers even preferring a completely blended sound, but nevertheless directionality is important implicitly. A composer necessarily writes with subconscious experience of the relationship between the sections of an orchestra, or between the different departments of an organ and the relationship of these to the choir; it has been said that the works of the Bach family cannot be fully appreciated except on an organ constructed on the *Werkprinzip*.

Some conductors pay considerable attention to the disposition of the sections of the orchestra, and these relationships should be heard in reproduction. There is little need, however, to pinpoint individual instruments, which indeed is seldom possible in the concert hall. What is important is that each voice in the musical texture should be labelled with an acoustic ambience characteristic of its place of origin. The ability to follow different voices in the musical texture, despite substantial differences in level, is very much bound up with this labelling, as indeed is the appreciation of musical timbre itself.

All these aspects relate to the acoustical reverberance and ambience of the concert hall. If it is worth spending thousands of pounds on the acoustics of a concert hall, then this should be heard in reproduction.

It follows that the highest fidelity in sound reproduction requires that the directionality of sound should mimic both the direct and reverberant sound of the concert hall. This is not just a matter of a vague splash of delayed echoes, but of a relationship between directionality and time-delay which gives specific information; this information is what we shall mean strictly by ambience. Systems capable of reproducing it will be called 'ambisonic'.

## 'Quadraphonic' attempt

In the recording industry it is customary to mix-down multi-microphone recordings onto four-track master tape in which signals are often stereo-blended between pairs of channels. The present upsurge of interest in surround-sound was in some measure triggered by engineers and producers playing back such four-track material directly into four amplifiers and loudspeakers distributed approximately in a square near to the corners of the monitor room.

Mixed-down tapes are unfortunately highly contrived and may have only the sketchiest relationship to natural sound. Moreover the assumption was that directionality should be encoded by pair-wise blending between channels associated with adjacent corner positions. This is a sub-optimal method of encoding, making less than full use of the information capacity of the available channels. Further, in order to achieve this coding using directional microphones it would be necessary for the polar response to have the highly improbable form of a half-cycle cosine wave over one sector of  $180^\circ$ , and zero response over the remaining  $180^\circ$ .

There are also severe restrictions in playback. If four loudspeakers are disposed about a listener, at least one pair must subtend an angle of  $90^\circ$  or greater at that listener. It is well established, however, that stereo blending does not work well when the angular subtense of the line joining the speakers exceeds about  $60^\circ$ . Moreover, stereo imagery works best over the  $90^\circ$  sector in front of the listener, worse at the rear, and worse still at the two sides. The attempt to extend the principle of stereo blending from the front wall to the other three sides of the listening room therefore not only retains all the limitations of stereo itself (outlined earlier) but is necessarily rather poor even judged as stereo.

The above approach is what is usually meant by the term 'quadraphony', although usage is by no means uniform. The name itself is a canard in at least two ways. First, it is a Greek-Latin hybrid, and ought to be called quadrasonic. The second is the identification, by implication, of surround-sound with the need to have four channels. In fact, as we shall see, surround-sound can be realised with as few as two channels. The false assumption that surround-sound is synonymous with four-channel then introduces the need to find distinctive names for two- and four-channel surround-sound systems, which were therefore called 'matrixed four-channel' and 'discrete four-channel', respectively. To prevent confusion I shall not use these jargon terms, and words such as four-channel and matrix will be used simply in their ordinary English and technical senses.

## More systematic approach

Any system that is to reproduce specific ambience information must embody the following (Fig. 1):

- (a) transduction of the original sound field, including its directionality and acoustic ambience;
- (b) encoding the resultant composite signal, including information about directionality, on to the available communication channels;
- (c) transmitting the resultant information to the listener, including recording and storage of this information where appropriate;
- (d) decoding the channel-signals into a form suitable for feeding to a plurality of loudspeakers;

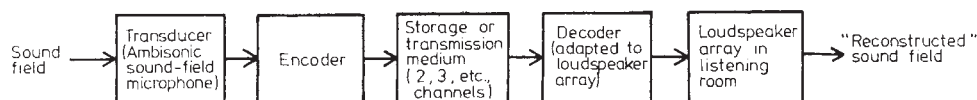


Fig. 1 Basic features of ambisonic reproduction.



- (e) radiation from the loudspeakers of sounds which interact in the listening space so as to reproduce, as far as possible, a simulacrum of the original sound field.

Our present systematic understanding of the requirements and possibilities of these steps owes much to the work of Gerzon<sup>2</sup>, particularly his analysis of the theory of encoding and of psycho-acoustic criteria in the reproduction of directionality, much of which is not yet published. The sections which next follow will discuss some of the salient problems in greater detail.

## Sound-field microphones

It is first necessary to characterise the sound-field at a given place in the room where the performance is taking place. The sound field at a point can conveniently be characterised in terms of spherical harmonics representing the sound field pressure and its derivatives. The number of spherical harmonics up to and including various orders  $n$  is shown in Table 1. From this it is seen that the number of independent signals, each representing a spherical harmonic, is of the form  $(n + 1)^2$ . The 0th order harmonic is equivalent to the sound-field pressure alone, and gives simply mono without directional information. The three spherical harmonics of order 1 are equivalent to the three cartesian components of particle velocity  $v_x$ ,  $v_y$  and  $v_z$ . Higher orders of spherical harmonics represent non-redundant combinations of higher gradients; they are of potential interest for the future but need not be considered for the purpose of current developments.

In order to deal properly with indirect sound, which may of course arrive from any direction, a sound-field microphone must encode in a deliberately designed manner any sound reaching it, including sounds having a vertical component of travel. It can be shown that microphones which violate this condition necessarily introduce undesirable coloration into the reproduction of reverberant sounds, even when the vertical information is deliberately suppressed in subsequent processing. From this requirement, having regard to Table 1, it follows that the number of independent signals generated by a microphone should correspond to the square of the natural numbers;  $(n + 1)^2 = 1$  giving monophonic reproduction, and  $(n + 1)^2 = 4$  the minimum uncoloured reproduction which includes directionality.

Table 1 Spherical harmonics

| Order | No. of harmonics | Cumulative total |
|-------|------------------|------------------|
| 0     | 1                | 1                |
| 1     | 3                | 4                |
| 2     | 5                | 9                |
| $n$   | $2n + 1$         | $(n + 1)^2$      |

Gerzon has shown that particular polyhedral arrangements of microphone capsules can fulfil the relevant requirements. The simplest of these places the capsules at the face centres of the regular tetrahedron. The spacing of the capsules should be kept as small as possible, but as it cannot practically be small compared with the wavelength at higher audio frequencies, correction circuits are needed to compensate for the resultant disturbance between the pressure and velocity responses of the composite microphone. There are some advantages in using a larger polyhedral array to generate a  $(n + 1)^2 = 4$  signal, and these are likely to be explored in the future.

The earliest ambisonic experiments were made using an improvised tetrahedral array of ordinary cardioid-response microphones. This arrangement, however, gives rise to considerable mutual interference, and it is impossible to obtain a sufficiently close spacing. Use of microphones in undesigned non-polyhedral arrays, or with spacings of 30 cm or more, or using replay direct into loudspeakers without pressure-velocity compensation, all give markedly sub-optimal results.

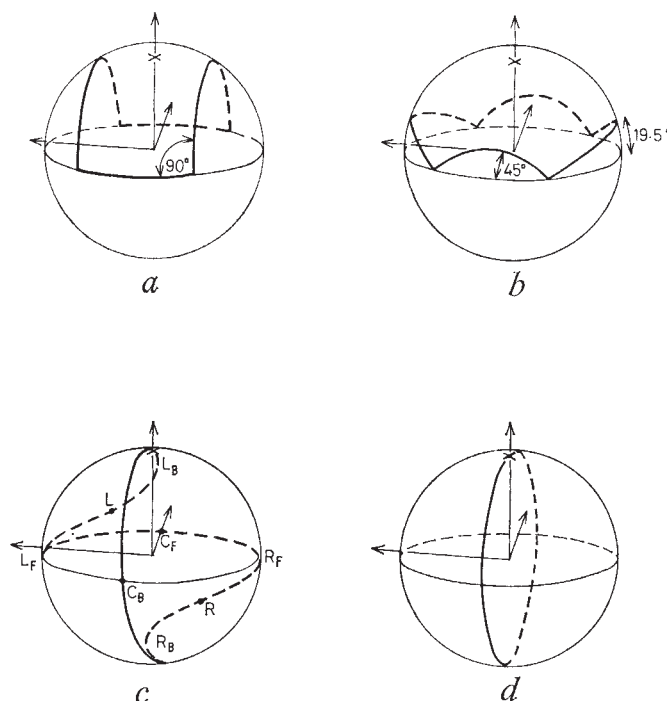


Fig. 2 Some representative horizontal pan-loci. *a*, Sansui 'QS'; *b*, closer approximation to great-circle locus consistent with four-channel master tapes (not in commercial use); *c*, CBS 'SQ'. Note cusps and left-right asymmetry due to choice of front-sector mapping and limitations of encoding from four pair-wise blended channels.  $C_F$ , Centre front;  $C_B$ , centre back;  $L$ , full left;  $R$ , full right;  $L_F$ , left front; and so on; *d*, a great-circle locus consistent with ambisonic encoding.

It is desirable to subject the raw signals from the sound-field microphone to a  $4 \times 4$  matrix transformation into a form, call **B**-format, in which they have reduced sensitivity to small phase or amplitude errors in transmission or recording.

## Encoding

The problem of encoding of directional information may be stated either in the form of how to make best use of a given number of available channels of communication, or of asking how many channels are necessary and sufficient for a given purpose.

Direction of travel can be characterised by the azimuth angle  $\theta$  and the altitude angle  $(\pi/2 - \phi)$ . Both parameters can be encoded on to only two channels carrying signals *A* and *B* for example as

$$|B|/|A| = \tan(\phi/2) \quad (1)$$

$$\angle B - \angle A = \theta \quad (2)$$

There are of course many other informationally-equivalent forms of these equations. In equation (1),  $\tan(\phi/2)$  is taken in order to avoid the 180° ambiguity that would result from using  $\tan\phi$ .

Equations (1) and (2) can equivalently be regarded as defining polar angles  $\theta$  and  $\phi$  (not necessarily identical with azimuth and altitude) which characterise the relative amplitude and relative phase of signals encoded on to two channels. Any given encoding can then be represented graphically by a point on a sphere, known as the Poincare or energy sphere<sup>3</sup>. This graphical representation is extremely useful in enabling the properties of proposed coding schemes to be understood, and it possesses a number of useful mathematical properties. The path on the energy sphere described by the representative point as the azimuth explores the horizontal circle is known as the horizontal pan locus; some representative examples are illustrated in Fig. 2.

There are several advantages in making this locus a great circle, and little or nothing to be gained by deviating from this form. Gerzon has shown that matrixing four pairwise-blended channels onto two channels, as in so-called quadraphony, necessarily gives a pan-locus consisting of four circular arcs intersecting so as to include a total angle of  $\pi$  rad at their corners; it therefore cannot give a great circle locus.

Encodings on to three or more channels can be represented similarly by points on hyper-spheres of appropriate dimensionality, but this is somewhat less useful as an aid to intuition because of the difficulty of graphical representation.

Equations (1) and (2) show that directionality can be completely encoded on to only two channels in the 'direction finding' sense, that is, any direction of arrival can be represented unambiguously. This is evidently necessary, but it is not sufficient, to ensure satisfactory performance of the overall ambisonic system, because it does not of itself ensure that the signal channels can be decoded in the required manner.

## Decoding and psycho-acoustic criteria

An exact replica of the original sound field, in the whole region occupied by the listener's head and up to the highest audible frequencies, would require many thousands of audio channels of communication and is quite impracticable. The closeness of approximation that can be achieved depends both on the number of channels and on the number of loudspeakers that are available.

At frequencies low enough for the human head to be small compared with the wavelength of sound, it is indeed possible to reproduce physically the original sound field. At higher frequencies this is no longer possible, and the best that can be done is to fulfil as many as possible of the psycho-acoustic criteria whereby sound directions are localised. These criteria, in relation to practicable systems, have been established particularly by the work of Gerzon already referred to.

Ambisonic reproduction in which vertical directional information is preserved may be called periphonic, and ambisonic reproduction with only horizontal information pantophonic. Pantophonic reproduction geometrically requires a minimum of three loudspeakers, since the triangle is the minimal figure able to enclose space in a plane. Similarly a minimum of four loudspeakers are geometrically necessary to surround the listener in three dimensions and give periphonic reproduction.

To satisfy the psycho-acoustic criteria sufficiently well, however, the practical minimum is four loudspeakers for pantophonic reproduction and six for periphony. One of the effects of using too few loudspeakers is that the loudspeakers themselves can be heard obtrusively as separate sources in addition to any ambisonic impression. This is closely associated with the 'detent effect' whereby as an actual source of sound moves its reproduced image seems to cling to each loudspeaker before jumping relatively rapidly to the next.

The limitations set by the number of loudspeakers interact with those set by the number of available channels. Qualitatively this is as expected, since the available number of loudspeakers limits the number of constraints available to control the sound field in the listening room, while the number of channels places restrictions on the extent to which these control constraints are independent. If four loudspeakers is taken as the practicable minimum for pantophonic reproduction, this is also in most people's minds at present the economic maximum. It can be shown that the capabilities of four loudspeakers in a planar array can be fully exploited using just three independent channels of audio information. By a happy coincidence, the current system of f.m. stereo broadcasting has sufficient bandwidth to transmit three audio channels. Interestingly, if the attempt is made to feed four loudspeakers from four channels in a non-redundant manner, the result is to enhance the detent effect. It can be shown that similarly four channels are sufficient to feed the minimal number of six loudspeakers required for periphonic reproduction. In general, the number of loudspeakers should

exceed the number of channels, so that it always pays to interpose a decoder between the communication channels and loudspeakers.

The practical outcome is that a two-channel four-loudspeaker pantophonic system can give good directional localisation with fulfilment of the salient psycho-acoustic criteria over a larger area than two-speaker stereo. Under favourable conditions, in surroundings that are acoustically neither too live nor too dead, the area of satisfactory and relaxed listening can fill most of an ordinary domestic room. The chief defect is a relative phase shift of  $180^\circ$  around the azimuth circle, unavoidable in any two-channel system, and the best that can be done is to distribute it in an optimally compromised manner. Current ambisonic decoders provide adjustments to compensate for spherical-wave effects of finite loudspeaker distance, and for non-square loudspeaker layouts.

The use of three channels enables the phase difficulty to be removed, and gives some improvement in the accuracy and stability of directional location. The next step in improving pantophonic reproduction is, as already indicated, to add additional loudspeakers rather than additional channels (which can actually make the results worse).Periphonic reproduction is, in the opinion of some who have heard it, at least as great an advance on pantophonic as the latter is on stereo, or stereo on mono. Three-channel periphonic systems may be possible with compromises not dissimilar to those involved in two-channel pantophonic reproduction. The minimum requirement of six loudspeakers in a non-planar array inhibits commercial interest at present, although periphony may have considerable interest for the future.

Beyond the  $(n+1)^2 = 4$  periphonic system, the next basis is  $(n+1)^2 = 9$ , which is also very much a matter for the future.

## Monophonic and stereophonic compatibility

The introduction of a new system always involves problems of compatibility with what went before. Ambisonic compatibility is therefore essentially a matter of the compatibility of its two-channel realisation with the existing two-channel mono and stereo formats.

Complete aesthetic compatibility is never possible between an old system and a new one providing additional information, as for example between stereo and mono or between colour and monochrome television. Technical compatibility depends on which out of a gamut of informationally equivalent ambisonic codings is employed. For ambisonic playback, these codings are equivalent because there is freedom to design the decoder to match the encoding. Ambisonic encoding can easily be made compatible with either stereo or mono, but not both simultaneously. A compromise is necessary, and the basic cause of this is not any fault in ambisonic encoding, but in the historical accident that the apparently obvious stereo and mono encodings concealed an inherent mutual incompatibility. Essentially, a balance must be struck between some attenuation of rear sources and some phase shift between the two loudspeakers in stereo playback; fortunately this can be done in a fairly harmless way.

Ambisonics is still in the experimental stage. It can be realised using two, three or more channels. The two-channel version has particular interest because of the wide availability of two-channel recording and broadcasting media developed originally for stereo use, and the three-channel version has particular interest in relation to f.m. broadcasting. Ambisonics is a complete system from the pick-up of the original sound field through encoding and decoding to the reconstruction of an approximation to the original sound-field in the listener's home. It therefore requires the simultaneous availability of both hardware and software; that is, of suitable decoders and of recordings or broadcasts on which to use them. None of these are commercially available at the present time, but may become so before very long.

Within the limitations of this article the attempt has been made to outline the basic principles of ambisonics with a minimum



of purely technical detail. Ambisonics is not so much a single system as a family of realisations having in common the attempt to state clear aesthetic and technological aims, to adjust these aims to what is physically and mathematically possible, and then to implement a properly engineered system to fulfil the objectives.

The development has been performed principally by M. Gerzon, J. S. Wright and the author with the help and support of the National Research Development Corporation. The

experimental equipment was built mainly in the Research Unit for Instrument Physics of the University of Reading. The help of numerous academic and industrial colleagues is also gratefully acknowledged.

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# Statistical physics, particle masses and the cosmological coincidences

J. K. Lawrence\* & G. Szamosi†

*A new look at some familiar 'cosmological coincidences' provides a means of 'predicting' appropriate mass values for the elementary particles.*

FOR many years, cosmologists have been intrigued by possibly coincidental relationships between physical constants of the microscopic and cosmic domains<sup>1</sup>. Examples of these are the approximate equality of such large dimensionless numbers as the ratio of the electromagnetic to the gravitational interaction strengths of particles, the ratio of the size of the Universe to the size of an elementary particle and the square root of the number of particles in the Universe. There has been controversy, not only over the possible meaning of these 'cosmological coincidences', but also over the existence of any meaning at all. Here we would like to show how, by means of simple assumptions about the nature of physical parameters and of the Universe, the cosmological coincidences may be derived in a logically coherent scheme. Equally important, this procedure yields a very reasonable limit for the mass of an elementary particle expressed in terms of fundamental constants. An extension of the procedure leads also to an expression for the fundamental electric charge.

The usual view of elementary particles is that similar particles are absolutely identical—that they have absolutely identical masses, electrical or nuclear interaction strengths, magnetic moments, and so on. A less restrictive view, and one perhaps more in keeping with our views of the physics of the early Universe, might be that the properties of elementary particles are determined stochastically. Thus, some physical parameters of a set of elementary particles, particularly their masses, might not be absolutely identical, but rather form statistical distributions about average values, which depend upon the overall thermal properties of the Universe. According to the usual principles of statistics, the standard deviation of the equilibrium mass would be

$$\delta m/m \approx 1/\sqrt{n} \quad (1)$$

where  $n$  is the number of particles in the Universe. Whether  $n$  is considered to be the total number of particles in the Universe or the number of a given species, such as electrons or nucleons, is not important within the accuracy considered here. In this same spirit, we regard the (average) masses of all elementary particles to be essentially equal.

## Variations of mass

We can now further restrict the mass fluctuations, using an anthropocentric argument similar to ones used in the past by

Dicke<sup>2</sup> and Carter in unpublished work. The existence of human life and awareness requires that the Pauli exclusion principle act over cosmological times. The nuclei of the various elements from which we are constructed must have existed for at least the age of the Solar System, and the structure of these nuclei depends on the Pauli principle, which depends on the indistinguishability of protons and of neutrons. If the masses of these nucleons differed by a detectable amount, the particles would no longer be indistinguishable and the Pauli principle would break down. According to quantum mechanics, in the age  $T$  of the Universe (or of the Solar System) it is possible to detect a mass difference  $\hbar/c^2 T$ . Thus we conclude that the mass spread of elementary particles must be less than this critical value. Then,

$$\delta m \approx \hbar/K_1 c^2 T \quad (2)$$

where  $K_1 > 1$  is a dimensionless constant.

We next make the assumption that the Universe, at least approximately, is just gravitationally bound. This allows us to relate the gravitational constant  $G$ , the mass  $M$ , the radius  $R$  and the age of the Universe by

$$R \approx cT \approx GM/c^2 \approx Gmn/c^2 \quad (3)$$

This relationship, sometimes connected with Mach's principle, is roughly compatible with observations and is widely assumed by cosmologists.

It is now possible to combine equations (1), (2) and (3) to obtain an expression for the mass of an elementary particle in terms of fundamental constants:

$$m \approx (\hbar^2/K_1 G c T)^{1/3} \quad (4)$$

Taking  $T \approx 10^{10}$  yr gives the numerical relation

$$m \approx K_1^{-1/3} \times 10^{-25} \text{ g} \quad (5)$$

This gives an upper limit for elementary particle masses which is consistent with the existence of human life in the Universe:

$$m \leq 10^{-25} \text{ g} \quad (6)$$

A wide range of values of  $K_1$ , covering many orders of magnitude, will give values of  $m$  lying within the range of elementary particle rest masses ( $\sim 10^{-27}$  g to  $\sim 10^{-24}$  g). A recent study by Reines and Sobel<sup>3</sup> indicates that the lifetime of the Pauli principle for inner shell electrons in iodine may be  $\sim T \times 10^{10}$  yr. Since the breakdown rate would be proportional to the square

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of the perturbation  $\delta m$ , this result implies a value  $K_1 \approx 10^5$  for these particles. This, when substituted into equation (5) implies  $m \approx 10^{-27}$  g, the electron mass. It would be of great interest to measure the analogous Pauli breakdown rate for heavier particles, such as nuclear protons or neutrons. A value  $K_1 \approx 1$  also gives a mass well within the range of known particle masses, and for convenience we will now adopt this value. It should be kept in mind that both the theory presented here and the corresponding observational results are very rough. Thus large changes in the value of  $K_1$  will have no fundamental effect on any of the following equations.

### Cosmological coincidence

Apparently, Weinberg<sup>4</sup> first noted the existence of equation (4) as a numerical coincidence and discussed its possible importance as a clue for a connection between microphysics and the Universe as a whole. The above considerations appear to give a possible rationale for its existence.

If we use  $K_1 \approx 1$  in equation (5), we find  $n \approx 10^{80}$ , which is compatible with present data, and  $\delta m \approx 10^{-65}$  g. By eliminating  $G$  from equations (3) and (4), we obtain

$$R/(\hbar/mc) \approx \sqrt{n} \quad (7)$$

Elimination of  $R$  yields

$$m \approx m_*/n^{1/4} \quad (8)$$

with  $m_* \equiv (\hbar c/G)^{1/2}$ , the Planck mass. Also one finds the gravitational fine structure constant

$$\hbar c/Gm^2 = (e^2/Gm^2)(1/\alpha) \approx \sqrt{n} \quad (9)$$

Equations (7) to (9) have often been noted in the literature as the 'cosmological coincidences.'

It is of further interest to consider the effect induced on particle self-energies by the variation  $\delta m$ . By requiring the induced variations in self-energy to remain too small for detection in the age of the Universe, as in the argument leading to equation (2), we obtain constraints on the values of the interaction coupling constants. We consider the formula for the lowest order electromagnetic self-energy of an electron given by Salam *et al.*<sup>5</sup> where the ultraviolet divergence has been removed by inclusion of gravitational self-energy:

$$E \approx (me^2c/\hbar)(\ln n) \quad (10)$$

Requiring the variation induced in this by  $\delta m$  to be undetectable gives for the fine structure constant

$$e^2/\hbar c \approx (K_1/K_2)/(\ln n) \quad (11)$$

Where  $K_2 > 1$  is another dimensionless constant. This result is roughly correct if  $K_2 \approx K_1$ .

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## Lightning in astronomy

E. W. Crew\*

*The author suggests that evidence for lightning on a grand scale in astronomy is most convincing. It might explain stellar flares, cosmic jets, quasars, galactic evolution, and much more. In this model, electrical charges accumulate in apparently highly conducting atmospheres.*

MOST of the characteristics of violent and sudden events<sup>1</sup> in astronomy can be explained simply in terms of well-established laws of physics if electrical discharges, similar to lightning, occur in the atmosphere of stars and galaxies. This entails the formation and separation of positive and negative charges of sufficient energy to produce electrical breakdown in gases which generally have far greater conductivity than the relatively cold and dense atmosphere of the Earth. Careful examination of the evidence for electrical discharges in astronomy shows, however, that these almost certainly do occur and therefore charging does take place. This was first suggested by C. E. R. Bruce in 1941, following extensive research on laboratory electrical discharges and lightning as a member of the Electrical Research Association.

I do not claim to conform with Bruce's views, but this article should give an indication of the scope and value of the work. Details have been published in many letters in scientific and technical journals, but the description of his theory as a whole, essential for understanding these letters, has had rather limited distribution, mainly in a report with full references<sup>2</sup>, a chapter in a popular science book<sup>3</sup> and a conference publication<sup>4</sup>.

The study of terrestrial lightning is full of uncertainties because of its unpredictable location and brief duration<sup>5</sup>. The immensely more powerful and prolonged discharges in astro-

nomy should help in the understanding of the processes in our atmosphere. The key to many astronomical events is the typical characteristic of an atmospheric discharge: a very rapid rise to maximum current, a steep decline, then a gradual fall over a relatively long period, with in some cases a secondary smaller rise of current, such as occurs in flare stars<sup>6</sup>.

### Solar characteristics

If electrical charges accumulate in the Sun's atmosphere until sudden breakdown occurs, the discharges and induced currents will produce all the high temperatures and ionised conditions which seem to most astronomers to prevent electrical charging. It is therefore advisable to examine more closely how charging could occur, instead of rejecting it on the basis of superficial appearances. The generation of charges is chiefly by the friction of glancing collisions between solid particles or liquid drops, much as in the Earth's atmosphere. Other processes such as splitting during solidifying and unequal size disintegration of drops with induced charges are probably also significant. Particles of high melting point metallic materials are formed at about 3,500 K and have been collected by rockets and Earth satellites<sup>7</sup>. The diffuse blue rings surrounding sunspots<sup>8</sup> are visible evidence of the formation of large numbers of small particles. They must also form in intergranular regions at the base of the photosphere and then be ejected into the upper regions by the violent winds and powerful thermals present.

Smaller particles are segregated from larger because forces

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acting on the projected area have greater effect on lighter particles. These generally have a preponderance of negative charge and therefore produce a surplus of negative electricity at high levels in the atmosphere, corresponding to a surplus of positive charge below<sup>9</sup>. The interactions of ions and free electrons with these particles is complicated, but it is likely that charges accumulate in the highly conducting atmosphere because the positive particles will rapidly capture free electrons. There will then be a surplus of positive ions which are far more massive than electrons and take much longer to neutralise the equivalent negative charge on the smaller particles, enabling the surplus negative charge to accumulate in regions remote from the positive charge zone. Hydrogen and other elements at this location and temperature are far from fully ionised<sup>10</sup> and the increasing voltage gradient due to the charges will eventually cause further ionisation, leading to electrical breakdown. The rate of charging will reduce as the voltage gradient increases, but the forces unleashed by solar activity are so immense that ample energy is evidently available for discharge conditions to be attained.

At any given time there are several million electrical discharges in the photospheric region, each about 1,000–2,000 km long and lasting 10 min, causing the average temperature of the solar 'surface' to rise to 6,000 K. The flow of positive ions in the electromagnetically compressed discharge channels persists for a time after the charge is consumed and the hot gas ascends and extends above the extinct arcs, forming the white patches of granulation. Most of the particles are recycled, as in terrestrial meteorology, but some are ejected to greater heights, aided by radiation pressure, carrying charge with them and building up electrical energy in the corona. The resulting discharges are generally more sporadic and violent, such as long prominences, and these are observed to have the same velocity of propagation as terrestrial lightning, attracting other prominences like currents in parallel conductors. The electrical and magnetic influence of these powerful discharges destroy some areas of the photospheric discharges, forming sunspots, in which the magnetic field pattern is the resultant of the surrounding photospheric discharges and that of the flare above. It is unnecessary therefore to assume that magnetic fields are produced by some unknown process inside the Sun, especially as the generally held view, that the magnetic field is always vertical in the umbra of sunspots, has been disproved by observations<sup>11</sup>.

The discharges in the perimeter of sunspots show as white streaks bent back by electromagnetic attraction towards the surrounding discharges. Jets from these discharges produce the outward flow of gas known as the Evershed effect, and their maximum observed velocity of  $8 \text{ km s}^{-1}$  agrees with Bruce's calculated value<sup>3</sup>. The photograph of a sunspot (Fig. 1) taken

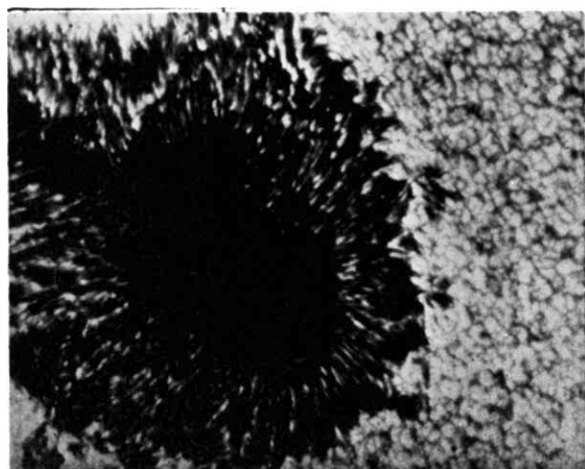


Fig. 1 Sunspot and granulation. (Courtesy Project Stratoscope of Princeton University.)

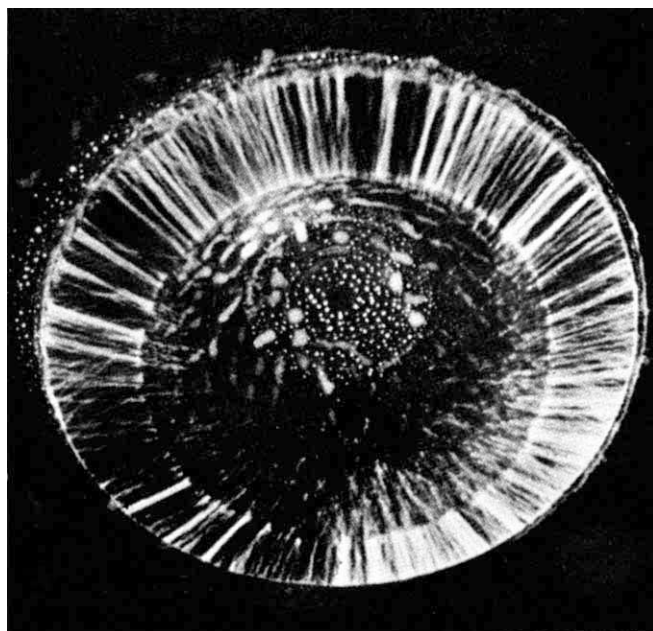


Fig. 2 Annular electrical discharges in a boiler igniter. (Courtesy Colt International Ltd, New Lane, Havant, Hampshire.)

by the Project Stratoscope<sup>12</sup> balloon-mounted telescope clearly shows the white tracks of electrical discharges of a width less than 300 km, the limit of the resolution of the telescope<sup>13</sup>. It is of interest to compare this with the photograph of an annular electrical discharge in a boiler igniter (Fig. 2), which shows several remarkable similarities, considering the enormous difference of scale. Many peripheral sunspot streaks resemble the jets produced by a laboratory plasma projector.

Bruce's theory explains many other solar characteristics in terms of electrical discharges and predicted temperatures of over  $10^8 \text{ K}$  in the corona a year before this was confirmed by observations<sup>14</sup>. He describes many cases of prolonged stellar atmospheric discharges, where the close correlation with observational evidence and similarity to lightning characteristics are most striking. It is an important confirmation of his theory that many planetary nebulae as well as galaxies have straight or spiral arms, evidently formed by discharges in which electromagnetic forces concentrate the matter in their channels. In planetary nebulae this material condenses to form binary partners and planets, while in the vast galactic discharge channels, stars and smaller nebulae are formed. The well known 'ring' type planetary nebula is only one of many different kinds, and if they all evolve in a similar way, then its shape is not that of an expanding shell of gas, but merely the result of a discharge jet happening to be in line, or nearly in line, with the observer. Doppler spectra characteristics show diametrically opposite jets in some of these nebulae.

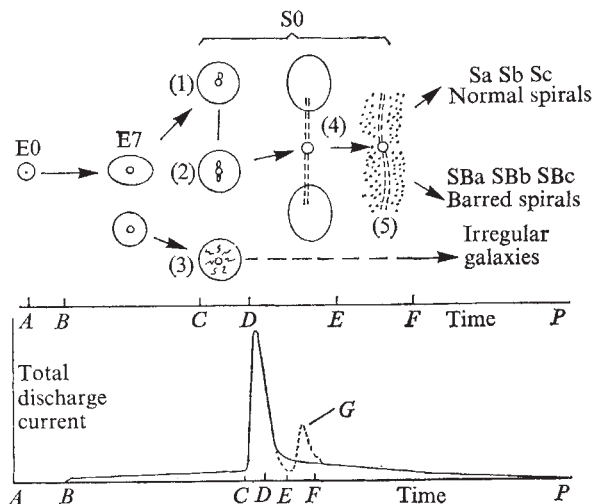
The theory that solar disturbances are caused mainly by the movements of charged atmospheric particles may explain why the level of activity in the Sun is so closely related to the tidal effects of the planets<sup>15</sup>. Small prominences and certain other solar features seem unlike any discharge process and may be caused by the varying magnetic fields of the large discharges acting on ions and charged particles, particularly when these are near the magnetic focus of deflected current channels.

### Galactic evolution

If charged particles are ejected or repelled by the hot nucleus of a galaxy for hundreds of millions of years (Hubble stages E0 to E7), a very extended charged atmosphere will develop with an immense store of electrical energy. The galaxy will then be poised on the brink of an enormous atmospheric discharge, in which a small increase in the voltage gradient would start a cataclysmic outburst near the nucleus and the jet would then advance far into the charged atmosphere. The initial main breakdown is likely to be initiated by the gravitational, electrical

or magnetic influence of an active neighbouring galaxy, possibly explaining why some discharge jets appear to point in that direction<sup>16</sup>. This 'quasar' phase occurs at Hubble stage S0, the point of departure for three types of evolution (Fig. 3). The discharge would very quickly attain peak output lasting for a few million years, during which time a second diametrically opposite discharge would occur, set off either by the extragalactic cause of the original discharge, or as a result of shock waves from the first jet orbiting the nucleus and focusing at the antipodes. Both discharges then continue to extend far into the surrounding negatively charged atmosphere at an average velocity of about  $4,000 \text{ km s}^{-1}$  (ref. 17).

Such a jet, with its myriads of discharge channels (similar to the few that are plainly visible in the great solar flare of June 4, 1946) would engulf a star like the Sun in minutes, heating the entire mass to thermonuclear temperatures and causing the short time variation of energy output which is observed in quasars. Magnetic pinch effects would also produce variations, but over far longer periods, as is evident from the jet of M87. An indication of diametrically opposite jets may be the difference in the values of the red shift of the nucleus, if detectable, which is the cosmological value, and the emission and absorption spectra of the jets, where there is a high relative velocity in line



**Fig. 3** Galactic evolution by electrical discharge processes. A-C, Long period of slow galactic growth ( $10^9$ – $10^{10}$  yr) and increasing temperature of its nucleus, which repels charged particles and starts the gradual accumulation of electrical energy. B-C, Atmospheric voltage gradient produces small aggregate leakage current (vertical scale exaggerated) which is limited by the availability of free ions in a very rarified atmosphere. When the maximum value is attained at any particular time and place, the voltage gradient can increase without a corresponding appreciable increase in the leakage current (Townsend effect). The gradient is highest near the nucleus and falls approximately as the inverse cube of the distance (for a distributed charge). C-D, Eventually the increase in the electrical field causes electrons to attain the energy required to produce further ionisation, and catastrophic discharge occurs. This is the quasar phase (1), lasting  $10^6$ – $10^7$  yr. During this time opposite jets form (2). In about 3% of galaxies irregular discharges occur (3). D-E, The extending discharges energise surrounding free electrons to relativistic velocities, which produce radio emission from extensive lobes (4). This is the radio-galaxy phase. E-F, The electrical charge rapidly diminishes and the lobes of radio emission contract. The regions surrounding the discharges cool and clouds of obscuring matter are produced. These absorb the energy of the high temperature core of the discharges and re-emit it as infrared radiation. This is the Seyfert phase (5). F-P, Charge continues to be collected from the galactic atmosphere, but the electromagnetically compressed discharge channels gradually cool and the matter condenses into the stars and planetary nebulae of the galactic arms. Some galaxies are distorted by impinging extragalactic fields. G, The main discharge may be restricted by the pinch effect, followed by another burst of energy at a lower level. Periodic outbursts (not illustrated) may be caused by repeated charge-discharge processes, helped by the huge forces of the active phase.

with the observer. The magnetic fields of the discharges extend to other galaxies, which frequently show the peculiar shapes caused by the impact of these powerful forces. Perhaps the reversals of terrestrial magnetism and other disturbances in our galaxy are the result of giant discharges in neighbouring galaxies.

The channel of a discharge would acquire a powerful positive charge, causing free electrons in the surrounding atmosphere to accelerate towards it, attaining relativistic velocities in extensive lobes on each side of the nucleus, as the quasar evolves into a radio galaxy (after Ryle<sup>18</sup>). Synchrotron emission by electrons in the extensive magnetic fields of the discharges would contribute to the total radiation. The flow of current following the characteristically 'brief' peak period of a few million years would be maintained by the collapsing magnetic field and the slower lateral current infeed from the surrounding charged zones. In straight discharges, matter from the jets then accumulates near the ends of the arms, as seen in NGC2859, but in discharges which are deflected by the field of adjacent galaxies or by rotation, the ejected matter continues in orbit about the nucleus, forming spiral arms. The intermediate Seyfert stage is illustrated in Fig. 3. A few cases are known where the spiral structure has apparently been formed by tidal forces. If random major discharges occur in the absence of a single main initial discharge due to an external influence or rotation, an irregular galaxy is formed.

The energy produced by the discharges is largely from thermonuclear reactions caused by high temperatures and electromagnetically increased pressures. No other known processes in astronomy can account adequately for the extent of nuclear synthesis required<sup>19</sup>. The energy was estimated by Bruce as  $10^{60}$  to  $10^{61}$  erg (ref. 2) on the basis of mass loss, which compared well with later estimates by others involving radiation measurements<sup>20</sup>. As in the stellar cases, many theoretical deductions by Bruce were confirmed by observations<sup>2</sup>. He has gone on to conjecture that strings of galaxies were formed as condensations in the universal discharge channels of the original Big Flash, and to predict that these galaxies have a higher content of heavy elements after their long subjection to thermonuclear reactions, compared with galaxies formed more slowly by gravitational aggregation outside the discharge channels.

### Validity of theory

Although in recent years many astronomers have suggested the time is ripe for a revolutionary new approach to clear up the many problems in astrophysics, few have commented on Bruce's work, presumably because it cannot be properly understood without some knowledge of the characteristics of lightning and other electrical discharges. Bruce was in the unusual and fortunate position of having studied these subjects intensively, his publications earning him a DSc. He then concentrated on the applications to astronomy, and was able to do this without preconceived ideas. His position outside the accepted astronomical organisations has, however, made it more difficult for his views to become widely known. One objection is that his theory is not supported by any detailed mathematical studies, but until it is better known and understood, these are unlikely to be attempted.

Perhaps this article will encourage workers in many fields of astrophysics to take a closer look at Bruce's work and to develop it in greater detail. If they will start by assuming it is possible for electrical charging and discharging to take place, then see where this will lead, perhaps the validity of their assumption would soon be established and C. E. R. Bruce would obtain the recognition he deserves.

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# articles

## The coenzyme-binding domains of glutamate dehydrogenases

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*A combination of secondary structure predictions and sequence comparisons with dehydrogenases of known structure allows two coenzyme-binding domains to be located in each of the sequences of bovine and Neurospora glutamate dehydrogenases. One domain shows significant sequence homology with the structurally-similar domain of glyceraldehyde-3-phosphate dehydrogenase.*

AMINO acid sequences of three glutamate dehydrogenases (GDHs) have been determined: the bovine liver<sup>1</sup> (500 residues) and chicken liver<sup>2</sup> (503 residues) enzymes, and recently the NADP-dependent enzyme from the fungus *Neurospora crassa*<sup>3</sup> (452 residues). All three are hexamers of identical subunits. Bovine and chicken GDHs are strongly homologous, differing only in 30 residues<sup>2</sup>, and although bovine GDH will be considered here the conclusions apply equally to the chicken enzyme. The *Neurospora* enzyme shows clear but limited homology with the vertebrate GDHs in the N-terminal two-thirds of the chain<sup>3,4</sup>, suggesting that the fungal and vertebrate enzymes shared a common evolutionary origin. The *Neurospora* GDH differs from the vertebrate enzymes in allosteric properties and in being NADP dependent. Vertebrate GDHs can utilise either NAD or NADP. Like many other microorganisms, *Neurospora* has a distinct NAD-dependent GDH which will not be considered here.

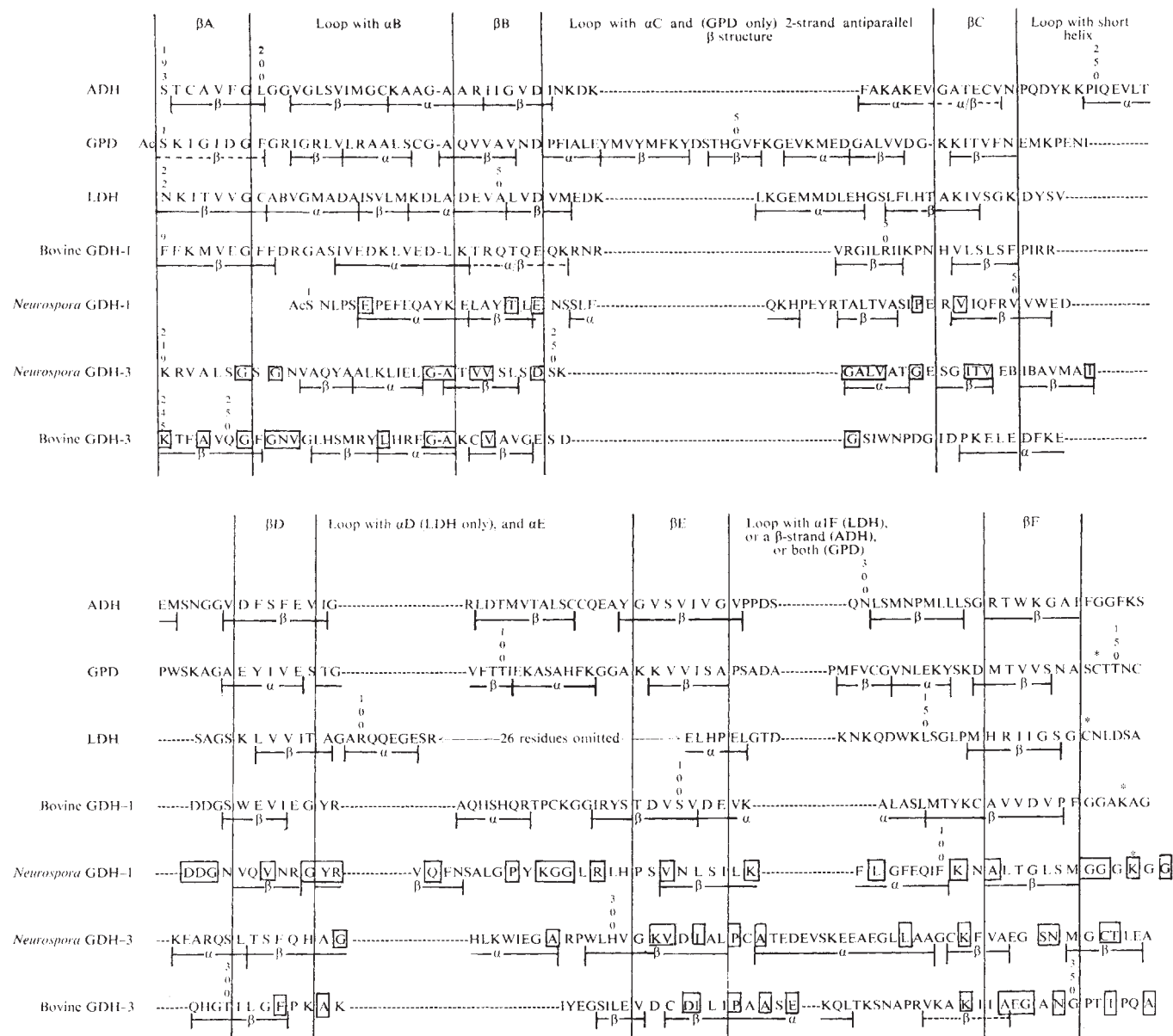
Knowledge of the three-dimensional structure of these enzymes is desirable but a crystallographic structure determination of any GDH is very remote. Large crystals and preliminary diffraction data have been obtained for the *Neurospora* enzyme (I. D. A. Swan, A. C. T. North and J. C. W., unpublished) but the crystal form examined had a large asymmetric unit and complex unit cell. As an interim measure I report here predictions of secondary structure in GDHs and sequence comparisons with other dehydrogenases of known structure. Taken together these two approaches allow the clear identification in each of the bovine and *Neurospora* GDH sequences of two structural domains (termed domain-1 and domain-3 because of their

positions in the sequence) which resemble the coenzyme-binding domains of other dehydrogenases.

The NAD-binding domains are of very similar structure in lactate dehydrogenase<sup>5</sup> (LDH), soluble malate dehydrogenase<sup>6</sup>, liver alcohol dehydrogenase<sup>7</sup> (ADH) and muscle glyceraldehyde-3-phosphate dehydrogenase<sup>8</sup> (GPD) and an analogous domain binds ATP in phosphoglycerate kinase<sup>9,10</sup>. A description and schematic diagram of this common domain is given by Rossmann *et al.*<sup>11</sup>. In different enzymes it varies between about 110 and 160 residues and contains a central six-stranded parallel  $\beta$  sheet. The  $\beta$  strands in their order in the sequence are termed  $\beta$ A,  $\beta$ B,  $\beta$ C,  $\beta$ D,  $\beta$ E and  $\beta$ F. The strand order in the  $\beta$  sheet in the above enzymes is CBADEF. The  $\beta$  strands are interconnected by more variable loops containing  $\alpha$  helices and in some cases  $\beta$  structure (Fig. 1). The ABC half of the domain is related to the DEF half by an approximate twofold axis, and each half has been termed a mononucleotide binding unit<sup>11</sup>. Domains with central parallel  $\beta$  sheet, some of which also have a nucleotide-binding function, have been described in other proteins<sup>11</sup>, but these differ from the dehydrogenase domains in the number or order of the  $\beta$  strands. Rossmann *et al.*<sup>11</sup> propose that all these domains share a remote common ancestor in precellular evolution.

### Secondary structure predictions

Of several available methods<sup>12–16</sup> for predicting the positions in protein sequence of  $\alpha$  helices and  $\beta$  structure, I have used the method of Chou and Fasman<sup>12,13</sup> which derives separate rules for the nucleation and stability of  $\alpha$  and  $\beta$  structures from the frequency of occurrence of residues in these structures in 17 proteins. In a comparison of several prediction methods applied to the parallel  $\beta$  sheet domain of adenylate kinase<sup>17</sup>, this method was as successful as any and compared well with the pooled results of the different methods. I have tested the applicability of the method to parallel  $\beta$  sheet domains by making predictions for the domains of known structure in ADH, GPD and LDH (Fig. 1), and by comparison of the number of residues found in the parallel  $\beta$  strands of six proteins with the numbers expected from the  $\beta$  potentials of Chou and Fasman<sup>12,13</sup> (Table 1). To simplify my report, predictions of bends will be omitted because they do not significantly affect the proposals for the GDHs.



**Fig. 1** Alignment of coenzyme-binding domains. The sequences (in one-letter code<sup>18</sup>) are of horse liver ADH (ref. 27), lobster muscle GPD (ref. 28), dog-fish  $M_4$  LDH (ref. 19), bovine liver GDH (ref. 1) and *Neurospora* NADP-dependent GDH (ref. 3). In the LDH sequence, 26 residues (not included in ref. 19) are omitted because different published versions conflict<sup>11,15</sup>. Except for some loop sequences the alignments of ADH, GPD and LDH are taken from ref. 11. Predictions of  $\alpha$ -helix and  $\beta$ -structure are indicated beneath each sequence. Dashed lines indicate ambiguities or tentative predictions (see text). Boxed residues emphasise the following sequence identities: in *Neurospora* GDH domain-1 with bovine GDH domain-1; in *Neurospora* GDH domain-3 with GPD; in bovine GDH domain-3 with *Neurospora* GDH domain-3. Precise residue-by-residue alignment can be deduced by counting residues from the nearest vertical line.

\* Reactive residues.

The overall frequencies of residues in parallel  $\beta$  sheets (Table 1) agree very well with expectation, the only significant deviations being an excess of asparagine (and perhaps also of valine and isoleucine) and deficiencies of glutamine and methionine. The distribution of residues along parallel  $\beta$  strands in these six proteins shows tendencies for lysine to favour the N-terminal ends and for glutamate and aspartate to favour the C-terminal ends. If the published  $\beta$  potentials<sup>12,13</sup> for lysine, glutamine, asparagine, glutamate and aspartate are modified to accommodate these discrepancies and tendencies, however, no  $\beta$ -prediction for the domains of ADH, GPD and LDH is altered by more than two residues. Therefore I have used the published  $\beta$  potentials without modification, taking—where given—the values based on 17 proteins<sup>13</sup> rather than those based on 15 (ref. 12).

I have also tried a less demanding nucleation condition to take into account the excess of valine and isoleucine: that any two of valine and isoleucine out of three residues is a possible

$\beta$  nucleus. This condition permits  $\beta$  predictions approximately corresponding to  $\beta$ A of GPD and to the putative  $\beta$ F of bovine GDH domain-3, which are not otherwise predicted as  $\beta$  strands, but this condition should not be applied generally without further tests. Predictions using this rule, and two ambiguous predictions, are indicated by dashed lines (in Fig. 1). The ambiguities arise in cases where two almost completely-overlapping sets of residues have high and almost equal average  $\alpha$  and  $\beta$  potentials. In other cases where predictions of high  $\alpha$  and  $\beta$  potential overlap, the residues have been resolved into an  $\alpha$  section (minimum lengths six residues) and an adjacent  $\beta$  section (minimum length five residues) provided these sections are individually consistent with the rules of Chou and Fasman<sup>13</sup>. The rules are somewhat ambiguous in defining the ends of  $\alpha$  and  $\beta$  sections and in some predictions in Fig. 1 up to two end residues could be added or subtracted arbitrarily.

The assignments for ADH, GPD and LDH (Fig. 1) show that the method is largely successful in predicting the approximate



**Table 1** Comparison of the numbers of residues observed in the parallel  $\beta$  strands of six proteins with the numbers expected from the  $\beta$  potentials of Chou and Fasman<sup>12,13</sup>

|       | Summed composition<br>of the six proteins | Number of residues found<br>in parallel $\beta$ sheet | Expected<br>number |
|-------|-------------------------------------------|-------------------------------------------------------|--------------------|
| Ala   | 140                                       | 19                                                    | 18.8               |
| Arg   | 41                                        | 4                                                     | 5.1                |
| Asn   | 51                                        | 10                                                    | 5.3                |
| Asp   | 84                                        | 9                                                     | 9.3                |
| Cys   | 27                                        | 4                                                     | 4.4                |
| Gln   | 46                                        | 1                                                     | 7.0                |
| Glu   | 88                                        | 7                                                     | 4.0                |
| Gly   | 157                                       | 19                                                    | 17.3               |
| His   | 27                                        | 3                                                     | 2.6                |
| Ile   | 88                                        | 25                                                    | 19.5               |
| Leu   | 114                                       | 13                                                    | 19.2               |
| Lys   | 124                                       | 17                                                    | 12.7               |
| Met   | 44                                        | 5                                                     | 10.2               |
| Phe   | 50                                        | 7                                                     | 10.6               |
| Pro   | 67                                        | 1                                                     | 4.2                |
| Ser   | 132                                       | 16                                                    | 14.6               |
| Thr   | 85                                        | 11                                                    | 14.1               |
| Trp   | 13                                        | 2                                                     | 2.1                |
| Tyr   | 37                                        | 6                                                     | 6.6                |
| Val   | 169                                       | 47                                                    | 38.5               |
| Total | 1,584                                     | 226                                                   | 226.1              |

The proteins, and the references to the structural information, are horse liver ADH<sup>7,27</sup>, lobster muscle GPD<sup>8,28</sup>, dogfish M<sub>1</sub> LDH<sup>5,19</sup>, porcine adenylate kinase<sup>31</sup>, *Peptostreptococcus elsdenii* flavodoxin<sup>32</sup> (aligned by homology with the *Clostridium* MP protein alignment<sup>11</sup>), and subtilisin Novo<sup>33</sup>. The expected numbers are calculated as described by Chou and Fasman<sup>12</sup>, using the summed amino-acid compositions of the six proteins (first column).

positions of  $\alpha$  and  $\beta$  sections, although the ends of the predicted sections are usually inaccurate by a few residues. Of the 18  $\beta$  strands in the parallel sheets the approximate positions for 14 (or 15 if the specific nucleation condition is used) are successfully predicted. Of the remaining three  $\beta$  strands,  $\beta$ C of ADH has an ambiguous rather than an incorrect assignment, and  $\beta$ D of GPD, although predicted as  $\alpha$  helix in the lobster sequence (Fig. 1) is predicted as  $\beta$  in the homologous pig and yeast enzymes. In the loops between the  $\beta$  strands many predictions are approximately correct, but in several cases part or all of some  $\alpha$  helices, particularly  $\alpha$ B and  $\alpha$ E, are predicted as  $\beta$ . The consistent occurrence of this type of incorrect prediction is interesting because it suggests that the stability of some of the  $\alpha$  helices may depend on long range constraints involving parts of the domain which are remote in the sequence. The general success of this and other prediction methods<sup>13,15</sup> is dependent on most secondary structure in the proteins being determined by short range interactions.

### Alignment of the GDH domains

Application of secondary structure predictions to the bovine and *Neurospora* GDH sequences revealed two candidates in each sequence for parallel  $\beta$  sheet domains. These resemble the known coenzyme-binding domains in being 100–150 residues long, very rich in secondary structure with a tendency for  $\alpha$  and  $\beta$  sections to alternate, in having candidates for bends only near the ends of  $\alpha$  and  $\beta$  sections and in the absence of long sections predicted as random coil. Secondary structure patterns of this type are not in themselves definitive of parallel  $\beta$  sheet domains because they could perhaps arise by chance or from other types of structure. Confidence in the conclusion that these parts of GDH are coenzyme-binding domains comes from the occurrence at approximately the correct positions of critical residues which are functionally important in coenzyme binding in other dehydrogenases<sup>11</sup>, and in the case of domain-3 from sequence homologies with the GPD domain.

The critical residues<sup>11</sup> used as criteria of alignment of the GDH domains are (Fig. 1, numbered for ADH), glycine 199, glycine 204 (conservatively replaced by alanine in *Neurospora* GDH domain-3), aspartate 223 (conservatively replaced by

glutamate in three of the GDH domains), and one or other of glycine 261 or glycine 270. Also used are the hydrophobic residues which occur in most but not all cases at the third and fifth positions from the N-terminus of each of the parallel  $\beta$  strands<sup>11</sup>.

Approximate alignments of the GDH domains were initially based on tentative identifications of the six parallel  $\beta$  strands from the secondary structure predictions, using the following criteria of the lengths of loops between the parallel  $\beta$  strands. First, the  $\alpha$ B loop is always 17 or 18 residues; second, the  $\beta$ C to  $\beta$ D loop is not shorter than 8 residues, as in LDH; third, no other loop is shorter than 10 residues. Twelve residues in the  $\alpha$ C loop of ADH is the shortest other loop of known structure, but ten-residue loops are structurally plausible.

Surprisingly, in the case of bovine GDH domain-1 only one approximate alignment was consistent with both the secondary structure predictions and the loop criteria, and this was slightly refined to align critical and hydrophobic residues, giving the alignment shown in Fig. 1. In this domain the  $\beta$ C,  $\beta$ E and  $\beta$ F regions may be inaccurate by a few residues, but no grossly-different alternatives satisfy the criteria. Minor adjustments could slightly increase the sequence similarities to ADH or GPD but at present these are not justified. *Neurospora* GDH domain-1 was aligned by sequence homology with bovine GDH domain-1. It lacks a candidate for the  $\beta$ A region but otherwise satisfies all the criteria well (Fig. 1).

In the case of domain-3 of both GDHs, several alternative approximate alignments, differing in the lengths of the loops, were equally consistent with the secondary structure predictions and loop criteria. Fortunately one of these alignments of *Neurospora* GDH showed sequence homology with the domains of the three GPD sequences, and the same alignment also conserved the critical and hydrophobic residues. These criteria give considerable confidence that this alignment of *Neurospora* GDH domain-3 (Fig. 1) is essentially correct, the only uncertainty

**Table 2** Sequence relationships between coenzyme-binding domains

|     |                     | GDH  |      |      |          |                     |                     |          |
|-----|---------------------|------|------|------|----------|---------------------|---------------------|----------|
|     |                     | ADH  | GPD  | LDH  | Bovine 1 | <i>Neurospora</i> 1 | <i>Neurospora</i> 3 | Bovine 3 |
|     | ADH                 |      | 12.8 | 9.5  | 9.6      | 2.9                 | 11.5                | 12.6     |
|     | GPD                 | 1.25 |      | 13.8 | 8.0      | 6.5                 | 20.1                | 14.4     |
|     | LDH                 | 1.30 | 1.21 |      | 6.7      | 5.1                 | 12.4                | 13.1     |
| GDH | Bovine 1            | 1.46 | 1.41 | 1.38 |          | 25.5                | 5.5                 | 9.6      |
|     | <i>Neurospora</i> 1 | 1.51 | 1.46 | 1.46 | 1.13     |                     | 8.1                 | 7.5      |
|     | <i>Neurospora</i> 3 | 1.36 | 1.16 | 1.22 | 1.45     | 1.36                |                     | 23.2     |
|     | Bovine 3            | 1.38 | 1.27 | 1.35 | 1.50     | 1.45                | 1.12                |          |

Pairwise comparisons of the alignments in Fig. 1 are expressed as (upper right of matrix) number of identical residues per 100 residues compared, and (lower left of matrix) minimum base changes per codon compared. Numbers for GPD are the average of the three comparisons with the lobster muscle<sup>28</sup>, pig muscle<sup>29</sup> and yeast<sup>30</sup> sequences, the latter two being unambiguously aligned by homology with the lobster GPD alignment in Fig. 1. Boxes enclose the comparisons showing strongly significant relatedness.

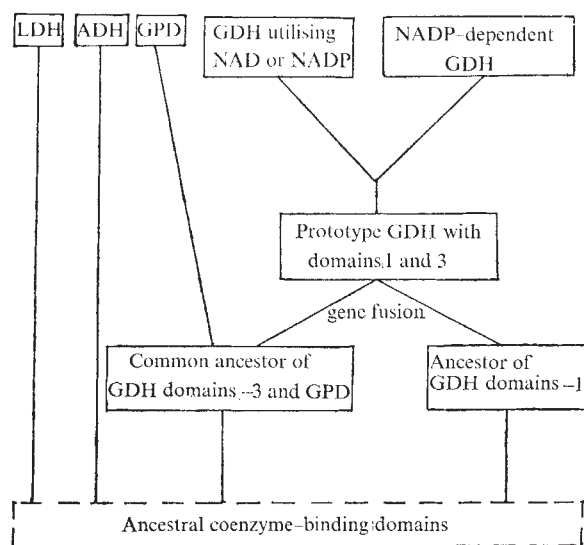


Fig. 2 A model of the early evolution of glutamate dehydrogenases.

being the exact location of the  $\beta$ D region where the sequence homology is weakest. Bovine GDH domain-3 was aligned by sequence homology with this *Neurospora* domain, and its alignment (Fig. 1) also conserves the critical and hydrophobic residues. The sequence identities in these alignments are emphasised by boxes in Fig. 1.

Part of bovine GDH domain-3 ( $\beta$ A to  $\beta$ B) was identified and aligned by Rossman *et al.*<sup>11</sup> on the basis of the sequence homology with GPD in this region, which includes the conservation of critical residues. In the bovine case this homology does not extend markedly to the rest of the domain. My arguments demonstrate that conclusions based on weak sequence homology can be considerably strengthened by secondary structure predictions which may in favourable cases provide a rational basis for the assignment of gaps to appropriate parts (such as loops) of sequence alignments.

## GDH domains and enzymology

Direct coenzyme-binding measurements<sup>20,21</sup> suggest that bovine GDH has two coenzyme-binding sites per polypeptide chain, one involved in enzyme activity with a high affinity for both NADH and NADPH, and the other of low affinity for NADH and very low affinity for NADPH. Similar measurements on *Neurospora* GDH (ref. 22 and B. Ashby, J. C. W., and J. R. S. Fincham, unpublished) have shown a stoichiometry of one molecule of NADPH bound per polypeptide chain in the conditions tried, but the inactivation of this enzyme by NADPH<sup>23</sup> is perhaps consistent with the presence of a second NADPH-binding site.

The alignment of domain-1 of the GDHs unexpectedly located the reactive lysine (126 in bovine, 113 in *Neurospora*) in the bend sequence immediately following  $\beta$ F (Fig. 1). In both enzymes<sup>24-26</sup> this lysine has an abnormally low  $pK$  and is highly reactive to pyridoxal-5'-phosphate. Modification of the lysine inactivates the enzyme and bound coenzyme protects against this inactivation. The reactive cysteines of LDH and GPD are in closely equivalent locations (Fig. 1), which places the reactive group close to the nicotinamide end of the bound coenzyme. In the equivalent bend of ADH lysine 323 occurs in a sequence similar to that of lysine 126 of bovine GDH (-Phe-Gly-Gly-X-Lys-). ADH lysine 323 is not known to be reactive. The reactive lysines of GDH could have either a catalytic role (as suggested for cysteine 148 of GPD (ref. 8)) or a location in which modification sterically interferes with coenzyme binding (as suggested for cysteine 164 of LDH<sup>6</sup>).

If GDH is analogous to LDH and GPD in the structural

relationship of the reactive residue to coenzyme binding, it follows that domain-1 is the site involved in activity. The alternative possibility cannot be excluded: that domain-3 is the site involved in activity and that the reactive lysine projects into domain-3 so that its modification interferes with coenzyme binding.

Although *Neurospora* GDH domain-1 lacks a sequence for  $\beta$ A and is most simply interpreted as a five-stranded  $\beta$  sheet domain, it is a good candidate for an NADP-specific binding site. Current evidence suggests that in this *Neurospora* GDH, the primary interaction in coenzyme-binding is with the 2'-phosphate of the adenosine ribose. NADH binding is not detectable (ref. 22 and B. Ashby, unpublished). 2'-AMP, but not 5'-AMP, is an inhibitor showing competitive kinetics with NADP (ref. 22), but the relatively low affinity for 2'-AMP suggests that interactions at the nicotinamide end of the coenzyme are also involved. It is possible that  $\beta$ A, which interacts with the adenosine ribose in NAD-binding domains, is dispensable in NADP-specific domains.

The putative domain-2, consisting of the sequence between domain-1 and domain-3, is the candidate for the functions of catalysis and 2-oxoglutarate or L-glutamate binding. It shows a slightly greater degree of sequence homology between bovine and *Neurospora* GDHs (ref. 3) than do domains 1 and 3. The possible domain-4, comprising the sequence from domain-3 to the C-terminus of the protein, shows no sequence homology and is completely different in secondary structure predictions in the bovine and *Neurospora* enzymes.

## Evolutionary implications

Table 2 shows the sequence relationships between the coenzyme binding domains aligned as in Fig. 1, expressed both as percentage of identical residues and as minimum base changes per codon. On either criterion three comparisons show a strongly significant genetic relatedness which provides evidence for a common evolutionary origin of the two sequences compared: bovine GDH domain-1 with *Neurospora* GDH domain-1, bovine GDH domain-3 with *Neurospora* GDH domain-3, and *Neurospora* GDH domain-3 with the domains of the three GPD sequences. Remarkably, *Neurospora* GDH domain-3 is almost as closely related to the GPD domains as it is to bovine GDH domain-3. The relatedness of bovine GDH domain-3 to the GPD domains appears much less strong because the homology is mainly restricted to the  $\beta$ A to  $\beta$ B region of the domain. The homologies between GDH and GPD suggest that the lines leading to the vertebrate and the NADP-dependent fungal GDHs diverged from each other very early in evolution, possibly at a precellular stage. A model for the precellular evolution of GDH is proposed (Fig. 2) in which domain-1 and domain-3 occurred initially on separate evolutionary lines and later joined by gene fusion at or after the time of divergence of GDH domain-3 from GPD.

Weakly significant relatedness also occurs between *Neurospora* GDH domain-3 and LDH, ADH and GPD, and LDH and GPD. This probably reflects a remote precellular common ancestry of all these domains<sup>11</sup>. Caution is required in the interpretation of weak sequence similarities because it is not known to what extent strong structural constraints on sets of residues can result in convergent evolution in different lines. Bovine and *Neurospora* GDHs domain-1, although strongly related to each other, are not significantly related to any of the other domains. This may reflect detailed faults in the alignments of Fig. 1, if these are out of phase in places with the true evolutionary alignments. Alternatively GDH domain-1 may have accepted mutations at a faster rate than other domains during the evolution of the utilisation of NADP.

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# Comparison of classical and autogenous systems of regulation in inducible operons

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*The mechanism of autogenous regulation, whereby a protein directly controls the expression of its own structural gene, is compared on the basis of function with the classical mechanism, in which the structural gene of the regulator is itself unregulated. The autogenous mechanism is superior to the classical one in inducible catabolic systems governed by a repressor; the opposite is true if the regulator is an activator.*

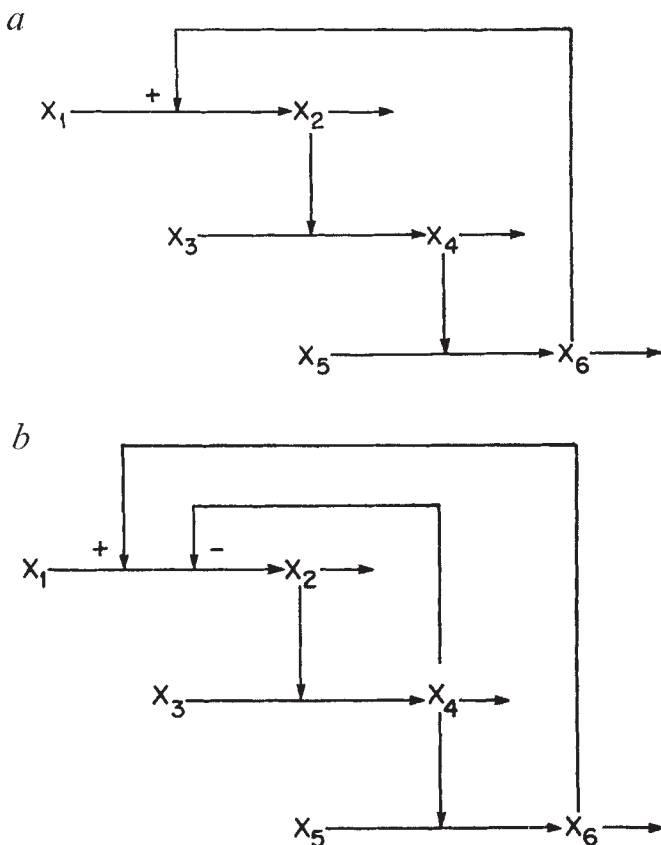
CONTROL of transcription by a constitutively synthesised repressor protein is central to Jacob and Monod's model for the regulation of gene expression<sup>1</sup>. Inducible operons were the first to be understood at this level of molecular detail in bacteria, and in several of the best studied examples, such as the lactose<sup>2</sup>, galactose<sup>3</sup> and glycerol<sup>4</sup> operons, the classical Jacob–Monod mechanism is believed to be operative. Alternatives, however, have been discovered: the regulator protein in some cases—such as the arabinose<sup>5</sup>, maltose<sup>6</sup> and D-serine deaminase<sup>7</sup> operons—is an activator, or positive element, in the control system. In other cases, for example, the histidine utilisation system<sup>8</sup>, there is a repressor mechanism but the regulator protein is directly involved in modulating the expression of its own structural gene. This latter feature of the *hut* system provides an example of a more general class of phenomena that has been called autogenous regulation<sup>9</sup>.

What are the functional implications of these differences? The relative merits of genetic control by repressors and activators have been explored elsewhere<sup>10</sup>. Here I shall examine the functional implications of control by a constitutively synthesised regulator and by an autogenously regulated one. To answer questions of this type one cannot compare directly two representative systems (for example, *lac* and *hut*) because they have numerous differences that are irrelevant to the comparison of classical

and autogenous regulation *per se*. A controlled comparison in which the two systems are identical in every respect except for the difference in regulatory mechanism is desirable but difficult to achieve experimentally. Nevertheless, such comparisons can be simulated by mathematical analysis. I have performed such an analysis comparing models of classical and autogenous regulation in inducible operons<sup>11</sup>. Before discussing the results I shall point out the criteria for functional effectiveness that I have used in comparing these systems.

## Criteria for functional effectiveness

To be specific, I will consider inducible catabolic systems in bacteria that allow the cell to utilise nutrients found in its environment. Several criteria can be formulated for functional effectiveness. (1) A sharp threshold in the concentration of the substrate necessary for induction protects the organism from wasteful synthesis of the catabolic enzymes when the substrate level is so low that insufficient benefit would be gained from induction. (2) The ability to make the most product available to the organism from a given supra-threshold increment in substrate is clearly advantageous to the organism whenever that substrate is the only nutrient available for growth. (3) Stability is obviously essential for a system to function properly. (4) A temporally responsive system can be induced rapidly, which is an advantage when substrate levels change abruptly. (5) Insensitivity to perturbations in the system's component parts ensures that the system will continue to function in spite of the continual perturbations it experiences in any real environment. These perturbations result from non-lethal mutations as well as physical changes in temperature and so on. None of these criteria assumes anything about the type of regulatory mechanism involved.



**Fig. 1** Schematic models of inducible catabolic systems. *a*, Classically regulated operon in which the structural gene of the regulator protein lies outside the operon and is regulated independently of the operon. *b*, Autogenously regulated operon in which the structural gene of the regulator is located within the transcriptional unit that the regulator controls.  $X_1$ , Nucleotide precursors;  $X_2$ , specific mRNA;  $X_3$ , amino acid precursors;  $X_4$ , enzymes of the catabolic pathway (plus regulator in the case of autogenous regulation);  $X_5$ , substrate;  $X_6$ , product-inducer. Although it is not critical for the comparisons reported here, the product has been chosen as inducer because in most cases the inducer is known to be a metabolic product rather than the substrate of the pathway<sup>12</sup>. The horizontal arrows indicate chemical transformations at the mRNA, enzyme and metabolite levels. The vertical arrows represent modifier or catalytic influences (the sense of the influence is positive unless otherwise indicated). Except for the differences in regulation these models are assumed to be identical.

## Comparison of repressor-controlled systems

First, I will compare inducible systems involving repressors that are regulated in the classical manner with those that are regulated autogenously. The two possibilities are represented schematically in Fig. 1. Except for the differences in regulation these models are assumed to be identical.

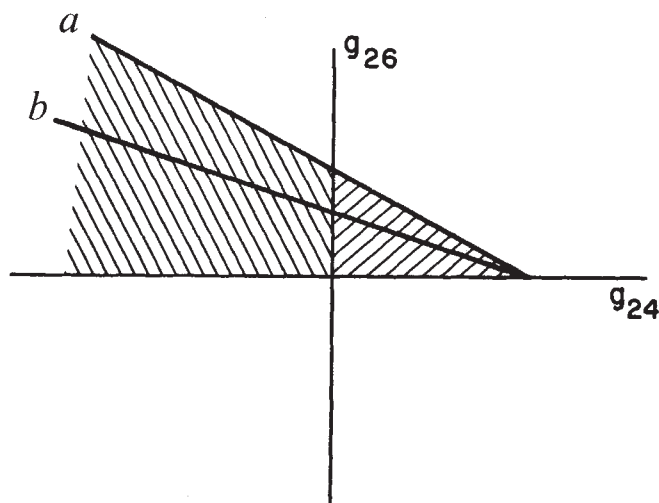
These two models can be compared under various conditions, and because there are several criteria to consider, a meaningful tabulation of all the results can present a problem. Figure 2, however, is a simple diagram summarising many of the results of these comparisons. This is a two-dimensional plot with the vertical axis representing the strength of the inducer's contribution to the control of transcription and the horizontal axis representing the strength of the autogenous contribution. Each point in this space can be thought of as representing a different system with distinct properties. This space, however, can be divided into subclasses of systems that have similar properties. Since I am comparing inducible systems that utilise a repressor mechanism, the inducer has a positive effect on transcription ( $g_{26} > 0$ ) while the autogenous effect is negative ( $g_{24} < 0$ ).

Thus, I can restrict my attention to the upper left-hand quadrant of this figure. The vertical axis in this quadrant is the locus of points representing classical systems, since the autogenous contribution is zero. Line (a) represents another classification. The position of this line is determined by analysing the stability of these models. All systems represented by points below line (a) are stable (if perturbed momentarily they will return to their predisturbance condition) whereas all those above this line are unstable (they will not return to their predisturbance condition).

Lines, such as (b), radiating from the point where line (a) intersects the horizontal axis, are lines of equivalence with respect to the first two criteria. All systems whose regulatory parameters have values that determine points on a given line, such as (b), have in common: (1) the same sharpness of threshold in substrate concentration for induction and (2) the same product formation for a given increment in substrate. The slope of the line is a quantitative measure of these properties. The steeper the slope is, the sharper will be the threshold and the greater the product formation.

Systems of the two types now can be compared. As noted before, all of the systems represented on a given line such as (b) behave identically according to the first two criteria. They differ, however, according to the third criterion. The position of the classical system is closer to the boundary of instability than are those of the corresponding autogenous systems represented in Fig. 2. In other words, line (b) and the boundary of instability, line (a), diverge to the left and the autogenous systems lying further from this boundary are more stable.

The fourth criterion, that of temporal responsiveness, is examined in Fig. 3. Curve (a) represents the response of the classical system. The system is in a steady state before  $t=0$ . At time zero the substrate concentration is suddenly increased to a new value that is maintained throughout the experiment. The concentration of the product is plotted as a function of time. Similar responses are plotted for a series



**Fig. 2** Graphical comparison of systems with alternative types of regulation. The vertical axis represents the strength of the product's contribution to the regulation of transcription and the horizontal axis represents the strength of the autogenous contribution to this regulation. Line (a) represents the boundary of instability. Line (b) is a representative line of equivalence with respect to the first two criteria for functional effectiveness. The vertical axis is the locus of points representing the classically regulated systems with either activator or repressor control. The shaded area to the left of this axis is the location of points representing stable systems with autogenously regulated repressors. Similarly, the shaded area to the right of the vertical axis represents stable systems with autogenously regulated activators. See text for further discussion.



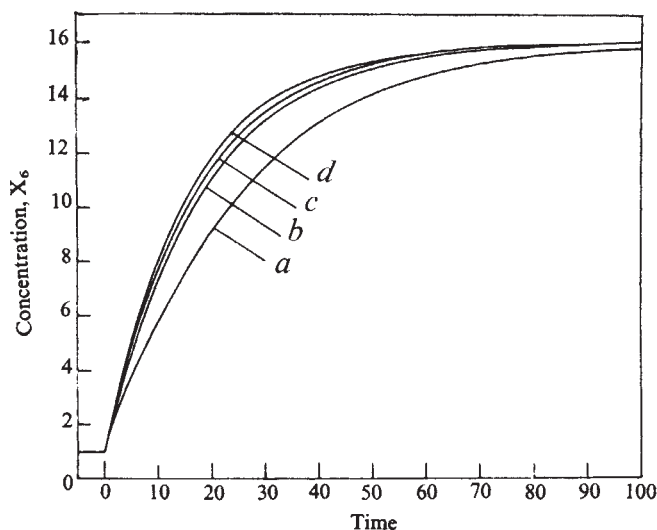


Fig. 3 Temporal responsiveness of equivalent systems with different strengths of autogenous regulation of repressor. The strength is represented by the parameter  $g_{24}$ , which has values:  $a$ , 0.0;  $b$ , -0.5;  $c$ , -1.0;  $d$ , -2.0. The corresponding values for the parameter  $g_{26}$  are chosen so that the points representing the different systems all lie on the same line of equivalence. Curve (a) represents the classical system with constitutively synthesised repressor. See text for further discussion.

of systems with increasing strengths of autogenous regulation and represented by points on the same line of equivalence (see Fig. 2). Systems with a high degree of stability tend to have more sluggish temporal responses to change. Figure 3 shows that the opposite relationship is true of systems with autogenous regulation of repressor.

Finally, a comparison on the basis of the fifth criterion shows that the sensitivities of the system with autogenous regulation of repressor to various perturbations in the structure of the system itself are always less than or equal to the corresponding sensitivities of the classical system.

These comparisons show that the autogenously regulated systems are equivalent or superior to the corresponding classical system according to all five criteria for functional effectiveness of an inducible catabolic pathway.

### Comparison of activator-controlled systems

Quite different results are obtained when classical and autogenous regulation are compared in systems governed by an activator protein. Schematic models representing these two types of systems are identical to those in Fig. 1, except that the negative sign is now positive, to indicate that the regulatory protein  $X_4$  has a positive effect on the transcription process. Again, except for the differences in regulation these models are assumed to be identical. The functions of these two models can be compared as in the previous section. Since the autogenous effect on transcription is now positive, I will restrict my attention to the upper right-hand quadrant in Fig. 2. The classical systems are represented along the vertical axis with zero autogenous contribution. Line (a) is the boundary of instability, so the stable systems of physiological interest here are represented by points within the shaded triangular area.

All systems represented on a line of equivalence such as (b) are equivalent with respect to the first two criteria. Since lines of equivalence, such as (b), and the boundary of instability, line (a), converge to the right, systems with increasing strength of autogenous regulation are represented nearer the boundary of instability than the corresponding classical system. Systems with autogenous regulation also

show a more sluggish temporal response to change, as Fig. 4 shows. Curve (a) represents the response of the classical system to an increase in substrate. The temporal responses are slower for the corresponding systems with increasing strengths of autogenous regulation (curves b and c). Finally, a comparison on the basis of the fifth criterion shows that the sensitivities of the system with autogenous regulation of activator are always greater than or equal to those of the corresponding classical system.

Thus, the results in this section are the opposite of those previously obtained for systems governed by a repressor protein. According to all five criteria for functional effectiveness, the classical system is equivalent or superior to the corresponding autogenously regulated systems.

### Predictions and observations

The functional differences I have discussed have obvious implications for the natural selection of classical and autogenous mechanisms of regulation in simple, inducible catabolic systems of the type represented in Fig. 1. Systems governed by an activator protein are not expected to utilise autogenous regulation. Activator-controlled systems without such regulation are superior to autogenously regulated but otherwise equivalent systems by all the criteria given for functional effectiveness. On the other hand, systems governed by a repressor protein can be predicted to involve autogenous regulation as well. Systems with autogenously regulated repressor are superior to classical but otherwise equivalent systems by all of these same criteria. How well do these predictions agree with experimental observations?

The first prediction agrees with what is known about activator-controlled systems in enteric bacteria. There is no known instance of autogenous regulation in such systems. In the best studied cases, the arabinose<sup>3</sup> and maltose<sup>6</sup> operons, the structural gene for the activator is located outside the transcriptional unit(s) known to be under its control. [The regulator protein of the *ara* operon is both an activator and a repressor. However, the predominant functional properties are those of an activator, and for our purposes the *ara* operon can be considered a (modified) activator-controlled system.] Thus, a simple, autogenously regulated operon is excluded. One could, of course, postulate more complex models of autogenous regulation in

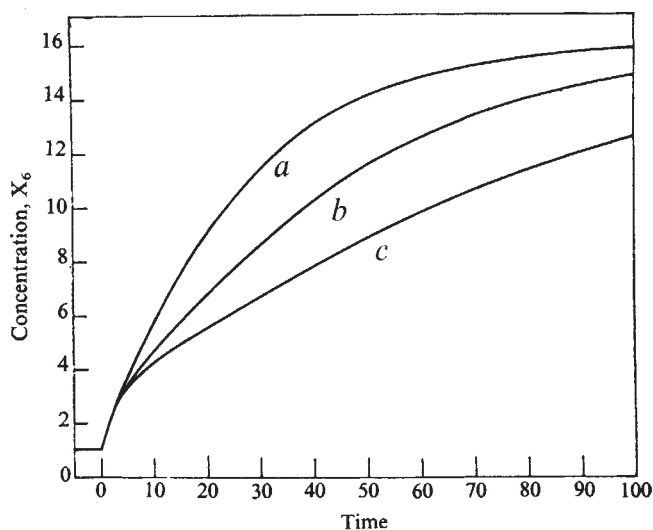


Fig. 4 Temporal responsiveness of equivalent systems with different strengths of autogenous regulation of activator. The strength is represented by the parameter  $g_{24}$ , which has values:  $a$ , 0.00;  $b$ , 0.15;  $c$ , 0.20. The corresponding values for the parameter  $g_{26}$  are chosen so that the points representing the different systems all lie on the same line of equivalence. Curve (a) represents the system with constitutively synthesised activator. See text for further discussion.

which the activator itself is autogenously regulated and its level varies with the expression of the structural genes in another transcriptional unit under its control. Functionally, this type of regulon model would be indistinguishable in most respects from the simple model in Fig. 1b. There is no evidence, however, to support or reject such a model.

The second prediction, concerning repressor-controlled systems, agrees only partially with available evidence. For the histidine utilisation system in *Salmonella typhimurium*, the evidence clearly agrees with the prediction of autogenous regulation. The structural gene for its repressor is located within one of the transcriptional units controlled by the repressor<sup>8</sup>. The expression of the second unit also varies with the level of the repressor<sup>13</sup>, which is functionally equivalent to autogenous regulation for most purposes, as previously indicated. There is no evidence for autogenous regulation in the other two well-studied repressor-controlled systems, the lactose<sup>2</sup> and galactose<sup>3</sup> operons. In each case, the structural gene for the repressor lies outside the only transcriptional unit known to be under its control, so that simple autogenous regulation appears to be excluded. This discrepancy might be explained in one of two ways. The systems might be regulated in a way that is functionally equivalent to autogenous regulation (one possibility has already been mentioned). Alternatively, the systems might have additional, as yet unknown, functions that are not reflected in the criteria for functional effectiveness and that require that the repressor not be autogenously regulated. The first explanation indicates that information about the molecular mechanisms is incomplete, while the second implies a lack of information about the physiological functions of these operons.

With regard to this last possibility, it is already clear that the *gal* operon is also a component of a much more complex system involved in the biosynthesis of capsular polysaccharide<sup>14</sup>. Recent evidence indicates that this operon is under the control of two repressors, specified by the *galR* and *capR* genes, respectively<sup>15</sup>. Thus, it would seem that the *gal* operon cannot be considered functionally as a simple, inducible catabolic system. Similarly, the *lac* operon

might yet be implicated in additional (dispensible) functions, possibly through the expression of the gene for the transacetylase enzyme, the function of which is presently unknown<sup>16</sup>.

It has been shown that autogenous regulation has certain advantages and disadvantages and that these have obvious implications for the natural selection of control mechanisms in inducible operons. There is reasonably good agreement of these predictions based on simple models of inducible catabolic systems with current observations of such operons in enteric bacteria. In those cases where the systems appear to be more complex, additional information about molecular mechanisms and physiological functions will be necessary before definitive comparisons can be made.

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# Seismic precursors before rock failures in mines

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*Seismic precursors (such as anomalous  $V_p$  values and/or seismic activity) whose behaviour is qualitatively similar to those reported to precede earthquakes are observed before rock failures in dry underground mines. Similar processes may be involved during failure of rock in the mine and the earthquake, and diffusion of fluids into the focal region of a potential earthquake may not be a necessary condition to produce most of the precursors reported.*

EVIDENCE is presented here that seismic precursor effects, such as anomalous  $V_p$  values and/or seismic activity, are observed before rock failures in underground mines. Seismic precursor information for rock bursts in a deep (5,000 feet) silver mine in northern Idaho and a roof fall in a Pennsylvania coal mine is discussed. These mines are dry and the effects of fluids on the seismic precursors are precluded.

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## Rock bursts at Galena

The Galena mine is located in the Coeur d'Alene mining district in northern Idaho. Silver, lead and zinc are the predominant metals mined from steeply dipping veins in the Belt formation series of Precambrian quartzites and argillites. A horizontal cut-and-fill mining system is used, with the mill tailings being pumped back into the mine to become the floor for the succeeding cut as mining proceeds from a lower level to a level 300 feet above. Detailed information relating to this type of mining system is available elsewhere<sup>1</sup>.

Rock bursts in the Galena have occurred in dry pillars at depths of 2,000 to 4,900 feet. In this article, rock bursts are qualitatively identified as small bursts (no damage to adjacent mine structures), bursts (no major damage to adjacent mine structures) and major bursts (substantial damage incurred to the adjacent mine structures). Past experience at the Galena has shown that the number of damaging rock bursts increases dramatically once the pillar thickness has been reduced to



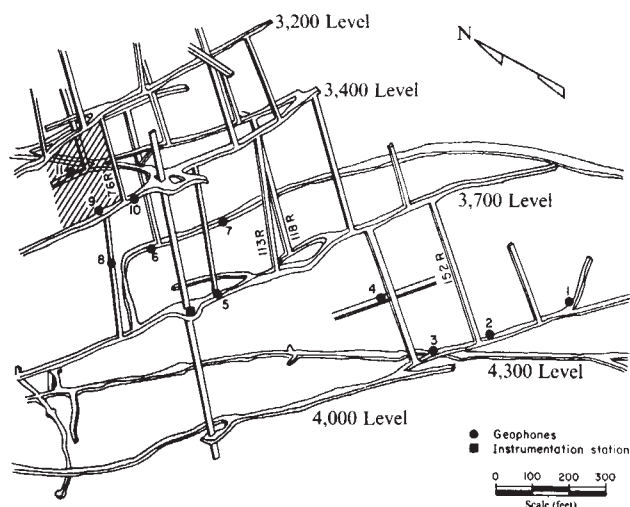


Fig. 1 Geophone locations and mine layout at the Galena mine<sup>2</sup>.

approximately 50 feet. Computer simulation of this mining method has also shown that the maximum principal stress reaches its peak value once the pillar thickness is reduced to 50 feet (ref. 2).

During 1968, detailed microseismic monitoring of potential rock burst zones at the Galena was begun by the United States Bureau of Mines<sup>2</sup>. To monitor a large part of the mine, geophones were placed on three different levels (3,400, 3,700 and 4,000 feet). Figure 1 shows the geophone locations (1-11) and the location of the monitoring station. Some of the results from one active zone (76R) are shown in Figs 2, 3 and 4. In Fig. 2, seismic activity, or equivalently rock noise count against time and pillar thickness, is indicated. The rock noise counts represent small rock failures in the immediate vicinity of the pillar. Note that typically little or no seismic activity is observed during the sandfill operation since the state of stress in the vicinity of the pillar is not changing. Cumulative plots of rock noise source locations are shown in Fig. 3. Seismic P-wave velocity ( $V_p$ ) variation with time is shown in Fig. 4. Seismic velocities were obtained for each day on which a blast occurred in 76R. These velocities allowed precise ( $\pm 20$  feet) location of rock noises. Accuracy of seismic velocities is estimated to be  $\pm 100$  feet  $s^{-1}$  (ref. 2). The P-wave variation with time in Fig. 4 for geophone stations 4, 5, 6 and 7 represent typical variations for all geophone locations.

Examination of Figs 2, 3 and 4 lead to the following observations. (1) The P-wave velocity increased to an apparently stable value for each geophone location once the pillar thickness was reduced to 48 feet. This result agrees with the observation that the maximum principal stress approaches a peak value once the pillar thickness is reduced to 50 feet (ref. 2). The P-wave velocity should also approach its maximum value at this time since

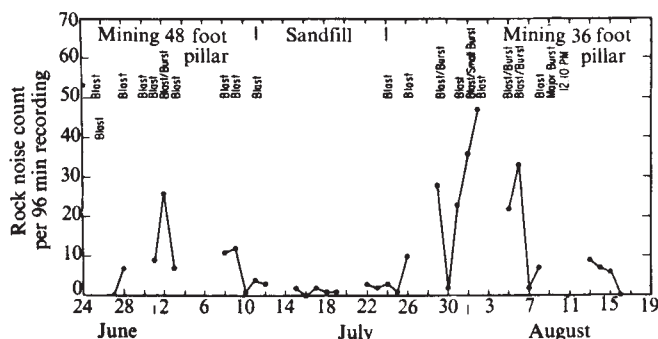


Fig. 2 Rock noise count plotted against time. (After ref. 2).

seismic velocities are known to be positively correlated with the maximum principal stress. (2) Before the major burst on August 9, the noise count in and adjacent to the pillar decreased distinctly. Noise counts resulting from the bursts of August 5 and 6 include noise counts from both zones. But when the source of each rock noise was located and separated into counts for each zone, the noise counts for the separate zones were found to decrease to low values before each of the bursts. The decrease

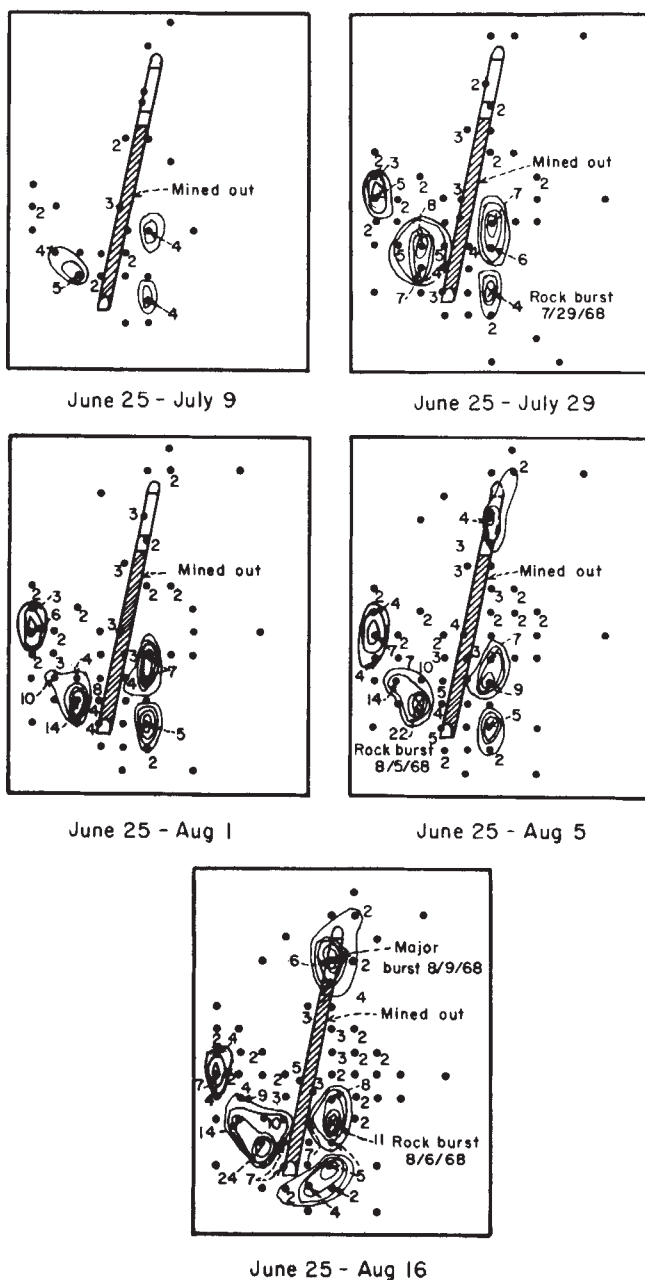


Fig. 3 Cumulative plots of rock noise source locations, 1968. (After ref. 2).

in each zone, however, was not as distinct as that observed for the major burst on August 9. No apparent decrease in noise count was observed before the small burst on August 1. These results suggest that the volume of rock involved in the burst may be an important factor in determining whether precursor phenomena, such as anomalous seismic activity, are observed before rock failures. (3)  $V_p$  decreases by approximately 6-8% before the rock bursts on August 1, 5, 6 and 9 shown in Figs 2,

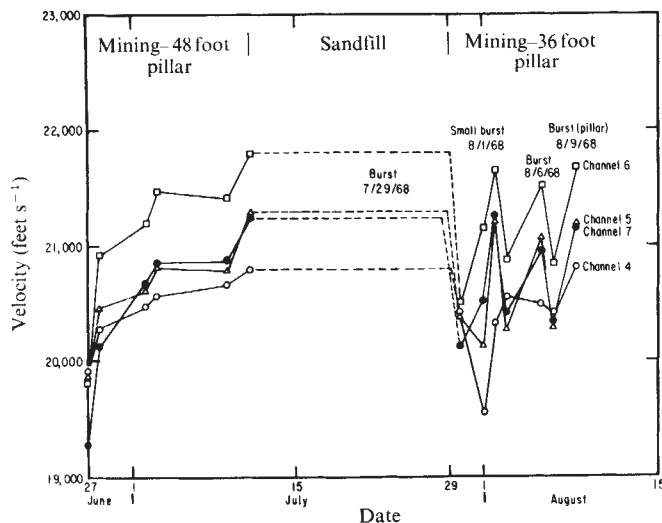


Fig. 4 P-wave variation with time and pillar geometry (76R).

3 and 4, and in general then regains its normal value, although the burst on August 6 did not occur when the velocity had returned to normal. This apparent exception may be a result of the effects produced by the burst of July 29 which occurred directly below the August 6 burst (Fig. 3). Note also that with the exception of the major burst on August 9, the other bursts occurred immediately after blasting.

The time intervals between the blasts in 76R are too long to attach much significance to most of the rock burst data in Fig. 4. It is significant, however, that the bursts of August 1 and 5 did occur when  $V_p$  had reached its normal value. The fact that these bursts occurred shortly after blasting precluded accurate determination of the time interval (precursor time) required for  $V_p$  to regain its normal value. This was not the case for the burst on August 9, which evolved independently of the possible effects produced by rapid load transferral as a result of blasting, which are reflected by the premature bursts on July 29, August 1, 5 and 6. It is important to note that before August 7, the noise count data suggest that there was a pronounced increase in stress within the pillar (Fig. 3). On August 8, however, there were few rock noises in the pillar even after blasting. The decrease in noise count in the pillar occurred at the same time that the P-wave velocity was increasing. This observation suggests that rather than releasing strain energy in the form of rock noises, the pillar had instead 'tightened up' and was storing strain energy. This effect could explain the observed increase in  $V_p$  and the decrease in the noise count before the burst. There are no data available on possible seismic activity that may have occurred in the pillar a few hours before the burst.

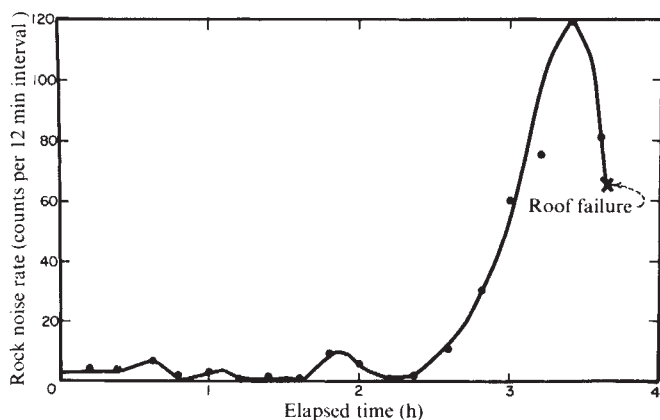


Fig. 5 Roof rock noise rate against time before roof failure.

## Coal mine roof fall

A recent application of microseismics is to provide an early warning of roof falls. The system used by the Bureau of Mines<sup>3</sup> had its first test during a controlled roof fall experiment conducted at the experimental coal mine of the United States Bureau of Mines at Bruceton, Pennsylvania. A room was first mined out and then widened in 0.31-m increments until the roof failed. The rate of emission of rock noise was monitored during mining. The results, illustrated in Fig. 5, show an anomalously high rate of rock noise emission beginning approximately 1 h before the failure. The noise count reached a maximum which

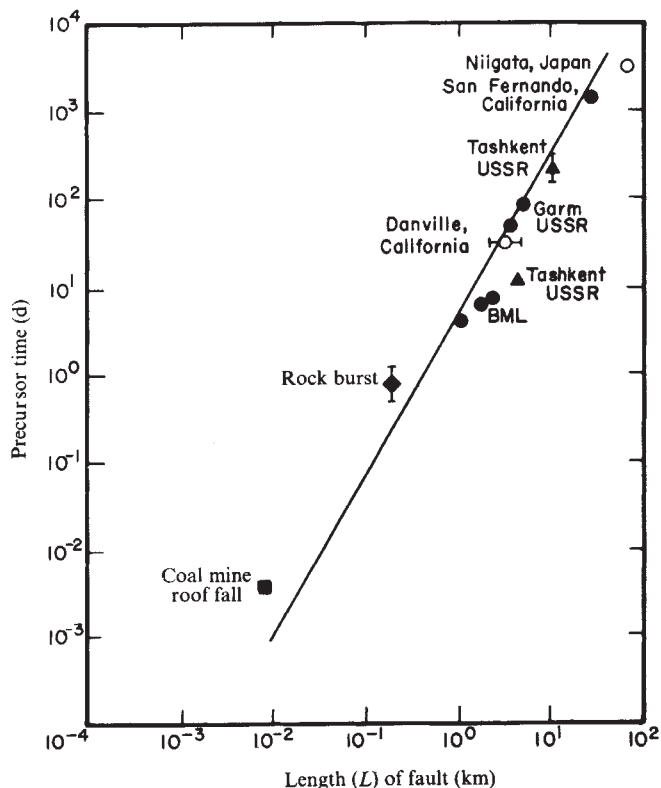


Fig. 6 Precursor time for several different failures as a function of source dimension. ○, Crystal movements; ▲, radon emission; ●,  $V_p/V_s$  anomaly; ◆,  $V_p$  anomaly; ■, noise count.

was then followed by a distinct decrease preceding the failure by about 10 to 15 min. The area of roof rock involved in the failure was approximately 70 m<sup>2</sup>.

## Implications

Observations at Garm (Soviet Union), the New York Adirondacks and for the San Fernando, California earthquake have shown that before these earthquakes the ratio of longitudinal ( $V_p$ ) and shear ( $V_s$ ) seismic velocities,  $V_p/V_s$ , decreased to anomalously low values and that earthquakes occur shortly following the return of  $V_p/V_s$  to its normal value<sup>4-7</sup>. These studies have also shown that  $V_p$  behaviour is the primary factor in the anomalous  $V_p/V_s$  behaviour. Anomalous behaviour in seismic rate (beginning before earthquakes) whose trend is qualitatively similar to that discussed earlier in mine failures has also been reported by Scholz *et al.* who reported that seismic activity decreased as the  $V_p/V_s$  value decreased before an earthquake of  $M = 2.5$  in the Blue Mountain Lake region. They also observed that the seismic activity did not increase as  $V_p/V_s$  subsequently increased but decreased further; just before the earthquake the activity increased. Some investigators have explained the behaviour of these precursor effects by requiring



the diffusion of fluids into the dilatant region of an impending earthquake<sup>4,6,7</sup>.

Precursor time ( $\Delta\tau$ ) versus 'effective length' ( $L$ ) of the fracture area for the August 9 burst and the coal mine roof fall are listed in Fig. 6. The precursor time for the rock burst was between 1 and 2 d. The effective length for the rock burst is approximately 100 m. This estimate is based on rock source locations following the burst. The effective length for the roof fall is approximately 8 m. (Both data points lie quite close to the general trend of  $\Delta\tau$  against  $L$  observed to hold for earthquakes.) The magnitude of the rock burst of August 9 was calculated to be  $M = 2.5$  based on data obtained from seismographs located in the mining district. The precursor time against magnitude relationship for this rock burst can be shown to follow the same relationship as that observed to hold for earthquakes<sup>6,7</sup>.

These data have two important implications. First, similar processes may be involved during failure of rock both in the mine and in the earthquake region; that is, rock failure is scale independent. Second, diffusion of fluids into the focal region of a potential earthquake may not be necessary to produce most of the precursor effects reported to occur before an earthquake since qualitatively similar precursor effects are observed in mines where no fluids are present.

I have developed a scale-independent failure theory governing the initiation and growth of shear faulting in dry rock that provides an explanation of why the precursor time-length-magnitude relationships hold for mine failures and earthquakes. A detailed account and implications of this theory will be published elsewhere<sup>8,9</sup>.

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# letters to nature

## Observations of the radio galaxy DA240 at 11 cm

THE radio source DA240 (refs 1 and 2) has been identified<sup>3</sup> as a 15-mag elliptical galaxy of redshift  $z=0.0356$ , placing it at a distance of 215 Mpc (assuming an Einstein-de Sitter cosmology with  $c/H_0=6,000$  Mpc). The total angular extent of the source is approximately  $34'$ , which implies a linear extent of 2.1 Mpc and makes it one of the largest systems known.

As part of a programme of source polarisation observations undertaken with the Effelsberg 100-m telescope, we have mapped the polarisation and total density distribution across DA240 at a wavelength of 11 cm with a resolution of  $4.8'$  (Fig. 1). The mode of observation and reduction has already been described<sup>4</sup>. The map was constructed from sets of right ascension scans. They did not, however, extend far enough to the west, so the base level of each scan was determined from the eastern extremity only and no gradient of base-level along the scan was removed. The map was not made under ideal atmospheric conditions, so that the limitation to the accuracy of the unswitched total intensity output is the accuracy of base-level determination. For example, a combination of several scans with a sloping base level is evident  $4'$  north of the map's centre at the 30 mJy level. The switched polarisation output is, in contrast, virtually unaffected by the weather, being only limited by noise at the 5 mJy level. We will discuss the three components of the source DA240 individually.

**North-east component.** At our resolution, the extended area is overshadowed by the strong compact component 4C56.16. The distribution of polarised flux is closely similar to the distribution of total flux over the central area of this component with a percentage polarisation close to 11% at a position angle of  $63^\circ$ . But on the southern edge the vectors swing around to position angles near  $150^\circ$  and the percentage polarisation rises to over 20%.

The percentage polarisation at 49 cm, both for the compact north-east component and the adjacent low brightness area, is 22% (ref. 3), which implies that there is little depolarisation through the source at this wavelength by differential Faraday rotation. The thermal electron density must, therefore, be very low. The differential rotation at 11 cm is 20 times less than at 49 cm but, nevertheless, the polarisation of the compact source and adjacent areas summed within our beam is only 11%. This is unlikely to be caused by differential Faraday rotation across the larger beam, either at the source, as the thermal electron densities there are so low, or in the Galaxy, as the cell size would need to be very small. Rather it must be caused by changes in the intrinsic position angle polarisation. Therefore, the magnetic field direction within the source changes significantly over distances of 1 to 2'.

**Central component.** At both 11 cm and 49 cm, the peak flux of the central compact component is a factor of 6 weaker than the peak flux of the north-east component. Over this wavelength range the two sources, therefore, have a similar spectrum (spectral index about  $-0.3$ ), flatter than the spectrum of the more extended components. But the difference in resolution of the two maps makes accurate comparison difficult. The compact sources have, however, quite different polarisation properties, for at 11 cm the polarised intensity associated with the central source is lost in the noise, indicating a percentage polarisation of less than 4%.

**South-west component.** Unlike the north-east component, the extended area in the south-west is not so dominated by a compact source, and fine scale structure in the polarised radiation is more clearly visible. Although the polarised flux is smaller than in the north-east, the percentage polarisation is higher: around 20 to 30%. Along the source axis, the position angle of polarisation swings from  $110^\circ$  near the centre to  $145^\circ$  about  $6'$  further out. Because this swing occurs over a distance of a little over one beamwidth,

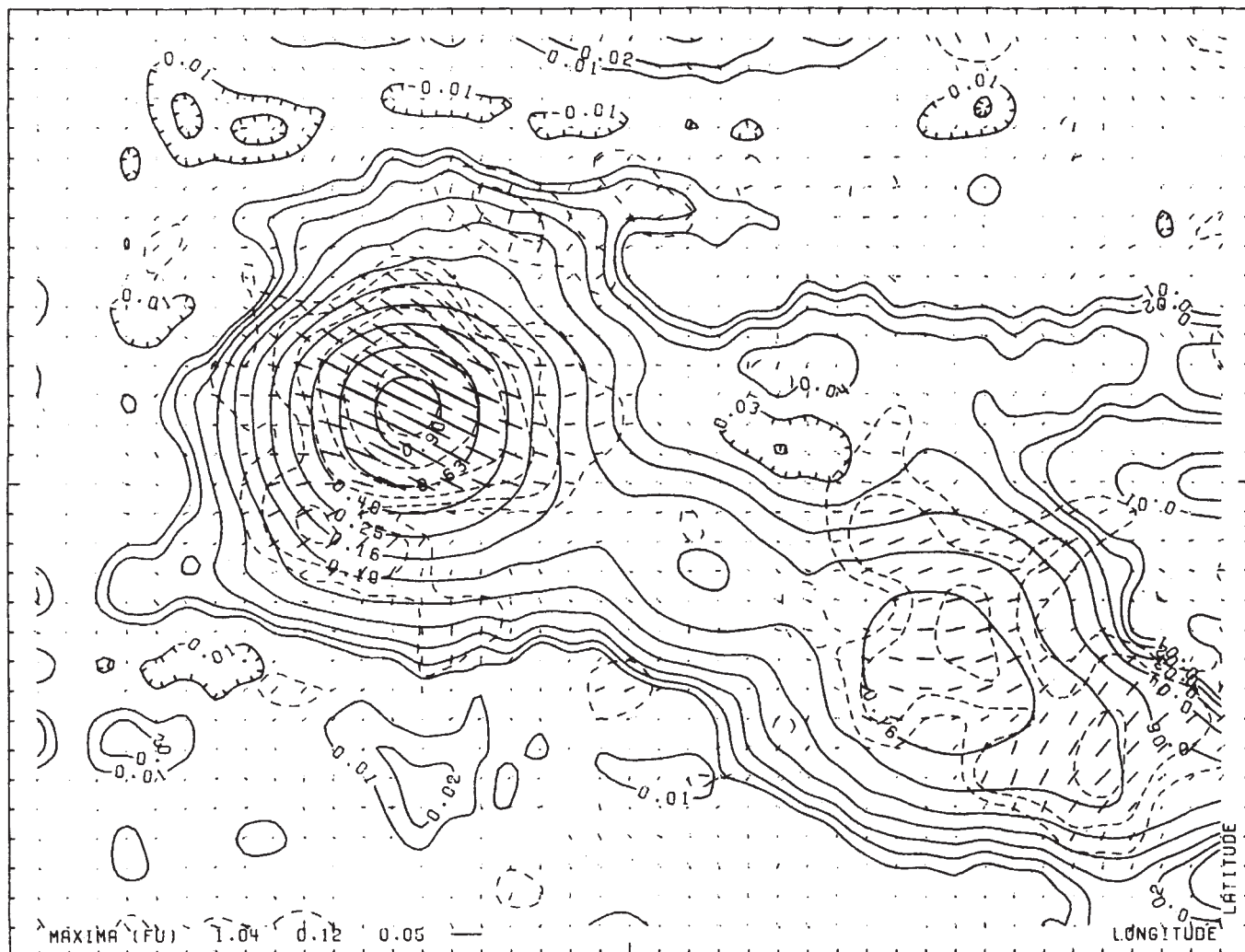


Fig. 1 DA240 at a wavelength of 11 cm. The map is centred on the position given by Bridle *et al.*<sup>2</sup>:  $\alpha(1950.0)=07^{\text{h}} 44^{\text{m}} 55^{\text{s}}$ ,  $\delta(1950.0)=55^{\circ} 58' 43''$  and covers an area of  $40'$  in  $\alpha$  (286 s time at this declination) by  $30'$  in  $\delta$ . The markers around the edge are at intervals of  $1'$ . The full contours represent the total intensity; eleven contours labelled to an accuracy of two decimal places, are plotted with a uniform logarithmic decrement of 2 dB from (1.00 down to 0.01) times the (north-east) peak intensity of 1,040 mJy, except that the top contour is depressed to the 0.90 level. The dotted contours represent the linearly polarised intensity. Six unlabelled contours are plotted from (1.00 down to 0.10) times the (north-east) linearly polarised peak intensity of 120 mJy, again with the top contour depressed to the 0.90 level. The vectors have a length proportional to the polarised intensity and are oriented along the electric vector of the polarised radiation. The maxima of the total intensity and polarised intensity distributions, together with the length of the unit polarisation vector, are indicated in the lower left hand corner. Longitude and latitude correspond in this plot to  $\alpha/\delta$  (1950.0).

vectors of different orientation are present simultaneously within our beam. This will not influence the position angle of polarisation, but does yield the apparent reduction in polarised flux evident on Fig. 1 between the two areas of maximum. If the magnetic field direction in the source did not change along the axis, this swing in the 11-cm position angles would require a change in rotation measure of  $50 \text{ rad m}^{-2}$ . Rotation measures of this order would yield high depolarisation at 49 cm, in conflict with the Westerbork observations. The rotation of the position angle of polarisation at 11 cm thus reflects a swing in magnetic field direction of  $\sim 35^{\circ}$  along the source axis over a distance of  $\sim 6'$ . Significant changes in magnetic field direction therefore seem to occur on a larger angular scale in the south-west component than in the north-east component.

Polarisation and total intensity measurements are planned at shorter wavelengths in order to delineate more precisely the changes of spectral index, rotation measure and particularly magnetic field direction across the whole of DA240.

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## A simple model of a Friedmann universe filled with a quantised scalar field

It is widely felt that the ultimate resolution of the problem of gravitational collapse awaits the formulation of a good theory of quantum gravity. Recently, however, it has been suggested (L. Parker and S. A. Fulling, unpublished) that singularities may be avoided because of quantum effects of the matter distribution rather than those of the gravitational field. When the radius of curvature of space-time becomes of the order of, say,  $10^{-13}$  cm one expects that the matter distribution might be more appropriately described by a quantum field than by its traditional description as a classical fluid. Since a quantum field need not necessarily respect the energy conditions required by the Hawking-Penrose singularity theorems, there exists, at least in principle, the possibility that in a gravitational collapse the radius of curvature of space-time may always remain much greater than the Planck length ( $10^{-33}$  cm) at which quantum gravitational effects are expected to become important.

Here I present a simple model related to the occurrence of singularities in a Friedmann cosmology. The model has the virtue that analytic solutions can be obtained which should provide qualitative insight into the results that may be available from a more complete theory.

The model is a 'mini-phase space' calculation in which a massive scalar field is quantised in the background geometry of a Friedmann universe. Coordinates may be chosen so that the line element in a Friedmann space assumes the form:

$$ds^2 = dt^2 - R^2(t) \gamma_{ij} dx^i dx^j \quad (1)$$

where  $\gamma_{ij}$  is the metric of a three-space of constant curvature. The equation of motion of a scalar field in these coordinates may be found from the Lagrangian density

$$\mathcal{L} = \frac{1}{2} g^{1/2} (g^{\alpha\beta} \varphi_{,\alpha} \varphi_{,\beta} - m^2 \varphi^2) \quad (2)$$

or equivalently from the action functional

$$\mathcal{S} = \int d^4x [\pi \dot{\varphi} - \frac{1}{2} g^{-1/2} \pi^2 + \frac{1}{2} g^{1/2} (g^{ij} \varphi_{,i} \varphi_{,j} - m^2 \varphi^2)] \quad (3)$$

in which  $\varphi$  and  $\pi$  are to be varied independently. The energy-momentum tensor for such a field is

$$T_{\mu\nu} = 2g^{-1/2} \partial \mathcal{L} / \partial g^{\mu\nu} = \varphi_{,\mu} \varphi_{,\nu} - \frac{1}{2} g_{\mu\nu} (g^{\alpha\beta} \varphi_{,\alpha} \varphi_{,\beta} - m^2 \varphi^2) \quad (4)$$

The problem may be simplified by restricting the phase space of the scalar field. I shall require that  $\varphi(x^i, t)$  be a function of the time  $t$  alone  $q(t)$  say.  $\pi(x^i, t)$  the momentum conjugate to  $\varphi(x^i, t)$  is a scalar density so I require it to be of the form

$$\pi(x^i, t) = \gamma^{1/2}(x^i) p(t) \quad (5)$$

Equation (3) now becomes:

$$\mathcal{S} = \int d^3x \gamma^{1/2}(x^i) \int dt \{ p \dot{q} - \frac{1}{2} R^{-3} p^2 - \frac{1}{2} m^2 R^3 q^2 \}$$

For the case in which the space is positively curved  $\int d^3x \gamma^{1/2}$  is a trivial multiplicative constant which we may drop and henceforward take:

$$\mathcal{S} = \int dt \{ p \dot{q} - \frac{1}{2} R^{-3} p^2 - \frac{1}{2} m^2 R^3 q^2 \} \quad (6)$$

In the cases of zero or negative curvature  $\int d^3x \gamma^{1/2}$  is infinite

though we may make it finite by restricting the range of the spatial coordinates and we shall adopt equation (5) in these cases also. From equation (6) we see that

$$p = R^3 \dot{q} \quad (7)$$

If we now restrict  $T_{\mu}^{\nu}$  to this mini-phase space, its non-zero components become

$$\rho \equiv T_0^0 = \frac{1}{2} (R^{-6} p^2 + m^2 q^2) \quad (8)$$

$$P \equiv -T_k^k = \frac{1}{2} (R^{-6} p^2 - m^2 q^2) \quad (9)$$

'Canonical' commutation relationships may be imposed, namely

$$[p, q] = -i$$

The Hamiltonian is tentatively identified from equation (6) as

$$\mathcal{H} = \frac{1}{2} R^{-3} p^2 + \frac{1}{2} m^2 R^3 q^2$$

Defining  $a$  by

$$a = [1/\sqrt{(2m)}] [R^{-3/2} p - imR^{3/2} q]$$

we see that

$$\mathcal{H} = (m/2)(a^\dagger a + aa^\dagger) = m(a^\dagger a + \frac{1}{2}) \quad (10)$$

We must now decide on the ordering of operators to be used in subsequent equations. This is necessary in order to determine correctly the source of the gravitational field. A natural way to do this is to order the operators so that the Hamiltonian becomes

$$\mathcal{H}_{new} = ma^\dagger a = \frac{1}{2} \{ R^{-3} p^2 + m^2 R^3 q^2 - m \} \quad (11)$$

This ensures that the lowest eigenvalue of  $\mathcal{H}_{new}$  is zero, this ordering also ensures the correct ordering of the Hamiltonian in the Minkowskian regime when  $\dot{R}/R$  is small.

The Schrödinger equation for the system is:

$$i \frac{\partial}{\partial t} \psi(q, t) = \mathcal{H} \psi(q, t) = \left\{ -\frac{R^3}{2} \frac{\partial^2}{\partial q^2} + \frac{m}{2} R^3 q^2 - \frac{m}{2} \right\} \psi(q, t) \quad (12)$$

where the radius function  $R(t)$  may be determined from the Einstein equations

$$G_{\mu\nu} = \langle T_{new\mu\nu} \rangle \quad (13)$$

once the normal ordered forms of  $\rho$  and  $P$  are known. Direct calculation from equations (8) and (9) yields

$$d(\langle \rho \rangle R^3)/dt + \langle P \rangle dR^3/dt = 0 \quad (14)$$

But it follows from (13), by the contracted Bianchi identities that

$$d(\langle \rho_{new} \rangle R^3)/dt + \langle P_{new} \rangle dR^3/dt = 0 \quad (15)$$

$\rho_{new}$  is determined from equation (11) by

$$\rho_{new} = R^{-3} \mathcal{H}_{new} = \rho - mR^{-3}/2 \quad (16)$$

Subtracting equation (15) from equation (14) then yields

$$P_{new} = P \quad (17)$$

The complete system of equations is now (henceforward all quantities are understood to be normal ordered)

$$\begin{aligned}
i\partial\psi/\partial t &= \frac{1}{2}(R^{-3}p^2 + m^2 R^3 q^2 - m)\psi \\
G_{\mu}^{\nu} &= \langle T_{\mu}^{\nu} \rangle \\
\rho &= \frac{1}{2}(R^{-6}p^2 + m^2 q^2 - mR^{-3}) \\
P &= \frac{1}{2}(R^{-6}p^2 - m^2 q^2)
\end{aligned} \quad (18)$$

We observe that although the energy density is a positive definite operator the pressure is not. Indeed the quantities  $\langle\rho\rangle + \langle P\rangle$  and  $\langle\rho\rangle + 3\langle P\rangle$  may be negative so that the 'strong energy condition' may be violated. This violation of the energy inequalities is not directly a consequence of the normal ordering prescription, since the strong energy condition is not satisfied even by a classical massive scalar field.

The exceptional case  $m = 0$  is trivially analysed, for then  $\langle\rho\rangle = \langle P\rangle \geq 0$ , the conditions of the singularity theorems are satisfied and a singularity ensues.

For  $m > 0$  it will be convenient to consider the cases  $k = -1, 0, +1$  separately. The Einstein equations may be written in the form

$$\begin{aligned}
6\ddot{R} &= -(\langle\rho\rangle + 3\langle P\rangle)R \\
\dot{R}^2 + k &= \frac{1}{3}\langle\rho\rangle R^2
\end{aligned} \quad (19)$$

When  $k = -1$ ,  $\dot{R}(t) \geq 1$  so that  $R(t)$  can have no minimum, hence  $R = 0$  at some finite time.

When  $k = 0$ ,  $R(t) \geq 0$ ; if  $\dot{R}(t_0) = 0$ ,  $R(t_0) > 0$  for some  $t_0$  then

$$\langle\rho\rangle|_{t_0} = \langle P\rangle|_{t_0} = 0$$

We make the assumption that solutions of equation (18) subject to appropriate initial data are unique. Then we must have a constant solution  $R(t) = R(t_0)$ , that is, we have Minkowski space-time. The same considerations obtain also when  $t_0 = -\infty$ .

When  $k = +1$ , there exist solutions which collapse to a singularity or bounce (as we go backwards in time) depending on the initial data.

To show that a singular solution exists we may construct a self-consistent asymptotic solution to the equations (18). Near  $R = 0$  we have

$$\psi_k(q, t) \sim \exp\left\{-\frac{ik^2}{2}\int^t \frac{ds}{R^3(s)} + ikq\right\}$$

with this wave function

$$\langle\rho\rangle \sim \frac{1}{2}k^2 R^{-6}, \quad \langle P\rangle \sim \frac{1}{2}k^2 R^{-6}$$

and hence by equation (19)

$$R(t) \sim [\sqrt{(3/2)kt}]^{1/3}$$

which has a zero at  $t = 0$ .

To construct a solution which has a local minimum in  $R(t)$  at  $t = 0$  we choose as an initial state

$$|t = 0\rangle = (1 + \lambda^2)^{-1/2}(|0\rangle + \lambda|2\rangle)$$

where  $|0\rangle$  and  $|2\rangle$  are the eigenstates of  $\mathcal{H}(t = 0)$  with eigenvalues 0 and  $2m$  respectively. At  $t = 0$  we have

$$\langle\rho\rangle = \lambda^2 2m R^{-3}/(1 + \lambda^2) \quad \langle P\rangle = \sqrt{2\lambda} 2m R^{-3}/(1 + \lambda^2)$$

If  $0 > \lambda > -3\sqrt{2}$  then  $\langle\rho\rangle + 3\langle P\rangle < 0$ . Choosing

$$R(0) = 2\lambda^2 m/[3(1 + \lambda^2)]$$

we have  $\dot{R}(0) = 0$  and  $\ddot{R}(0) > 0$  so that  $R(t)$  has a local minimum at  $t = 0$ .

From equation (18) we see that for  $R(t) \ll m^{-1}$  the equations approach, in some sense, those obtaining for zero mass for which collapse is inevitable. For any  $\varepsilon > 0$  a set of solutions of non-zero measure satisfies  $R(t) < \varepsilon m^{-1}$  for some  $t$ , and for sufficiently small  $\varepsilon$  a subset of non-zero measure of these will collapse. Thus we see that the set of solutions which exhibits a singularity is of non-zero measure.

The results of this calculation indicate that the introduction of a quantised matter source into the Einstein equations does not cause the system to 'conspire' to evade a singularity. It is of interest to note that the results of this calculation are in accord with the results obtained by L. Parker and S. A. Fulling, and W. F. Blyth and C. J. Isham, unpublished. I acknowledge fruitful conversations with Dr D. J. Raine and thank the Science Research Council for financial support.

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## Origin of fifteen cosmic dust particles intercepted by Pioneer 8 and 9

Two identical cosmic dust particle experiments on board the heliocentric space probes Pioneer 8 and 9 have now yielded consistent data for more than 8 yr. The two spacecraft are in direct heliocentric orbits with perihelia between 0.75 AU and 1.00 AU and aphelia between 1.00 AU and 1.10 AU, respectively. Their orbital velocity is approximately 30 km s<sup>-1</sup> and both satellites are spinning at 1 r.p.s. about an axis perpendicular to the ecliptic plane, and carry similar cosmic dust detectors scanning the ecliptic plane<sup>1-4</sup>. The instrumentation, its calibration, and significant scientific results have been reported<sup>1-3</sup>.

We are primarily concerned with the time-of-flight data which provided orbital characteristics of small interplanetary dust particles in the solar system. Out of the total 20 events recorded to date, 5 show hyperbolic characteristics and are excluded from this specific study. The semimajor axes and eccentricities for the remaining 15 elliptical orbits are shown in Table 1.

There are two general criteria one can apply to determine the origin of these dust particles. Whipple has introduced an empirical relationship to distinguish cometary particles from asteroid objects<sup>5</sup>, referring to it as the  $K$  criterion defined as

$$K = \log [a(1+e)/(1-e)] - 1 \quad (1)$$

where  $a$  is the semimajor axis in AU and  $e$  is the eccentricity.  $K$  is positive for the majority of cometary orbits and negative for the majority of asteroids. This  $K$  criterion has no basic physical significance, except as a useful tool in any discussion of the general characteristics of the meteor orbits and their origin. Computed values of  $K$  for the Pioneer 8 and 9 events are shown in Table 1. According to the  $K$  criterion, eight particles are definitely asteroidal and two are cometary. The remaining five events are intermediate and uncertain. Computed Tisserand invariant  $T$  also shows that with the exception of event 14, all of them have  $T$  greater than 1.00 and thus align with the Apollo group<sup>6</sup>. The 15 elliptical orbits have small semimajor axes, small perihelion distances, and moderate eccentricities,



**Table 1** Classification of 15 cosmic dust particles detected by Pioneer 8 and 9 experiments

| Event Number | Semimajor axis (a) (AU) | Eccentricity (e) | K     | Type         |
|--------------|-------------------------|------------------|-------|--------------|
| 1            | a                       | 0.864            | 0.226 | Asteroidal   |
|              | b                       | 0.830            | 0.264 |              |
|              | c                       | 0.816            | 0.301 |              |
| 2            | a                       | 0.623            | 0.729 | Asteroidal   |
|              | b                       | 0.601            | 0.791 |              |
|              | c                       | 0.585            | 0.844 |              |
| 3            | a                       | 0.786            | 0.850 | Intermediate |
|              | b                       | 0.812            | 0.905 |              |
|              | c                       | 0.854            | 0.946 |              |
| 4            | a                       | 0.561            | 0.990 | Intermediate |
|              | b                       | 0.616            | 0.886 |              |
|              | c                       | 0.763            | 0.624 |              |
| 5            | a                       | 0.675            | 0.626 | Asteroidal   |
|              | b                       | 0.652            | 0.685 |              |
|              | c                       | 0.633            | 0.738 |              |
| 6            | a                       | 1.010            | 0.090 | Asteroidal   |
|              | b                       | 0.977            | 0.101 |              |
|              | c                       | 0.950            | 0.118 |              |
| 7            | a                       | 0.979            | 0.256 | Asteroidal   |
|              | b                       | 0.969            | 0.275 |              |
|              | c                       | 0.960            | 0.295 |              |
| 8            | a                       | 0.830            | 0.544 | Asteroidal   |
|              | b                       | 0.832            | 0.593 |              |
|              | c                       | 0.830            | 0.639 |              |
| 10           | a                       | 0.541            | 0.999 | Cometary     |
|              | b                       | 0.602            | 0.966 |              |
|              | c                       | 0.757            | 0.866 |              |
| 12           | a                       | 1.13             | 0.593 | Asteroidal   |
|              | b                       | 1.16             | 0.645 |              |
|              | c                       | 1.22             | 0.695 |              |
| 13           | a                       | 0.555            | 0.993 | Intermediate |
|              | b                       | 0.608            | 0.875 |              |
|              | c                       | 0.748            | 0.840 |              |
| 14           | a                       | 0.962            | 0.801 | Intermediate |
|              | b                       | 1.620            | 0.827 |              |
|              | c                       | 5.510            | 0.992 |              |
| 15           | a                       | 0.545            | 0.993 | Cometary     |
|              | b                       | 0.545            | 0.998 |              |
|              | c                       | 0.553            | 0.973 |              |
| 17           | a                       | 0.719            | 0.861 | Intermediate |
|              | b                       | 0.720            | 0.927 |              |
|              | c                       | 0.735            | 0.970 |              |
| 19           | a                       | 0.744            | 0.519 | Asteroidal   |
|              | b                       | 0.712            | 0.586 |              |
|              | c                       | 0.690            | 0.643 |              |

Listed as numbered by Berg *et al.*<sup>2</sup>.

Due to uncertainties in the calibration curve (typically velocities:  $\pm 18\%$ , inclinations:  $\pm 27^\circ$ ), three sets of possible orbital parameters are shown for each event and represented as a, b, and c. The particle mass density has been assumed to be  $3 \text{ g cm}^{-3}$  in this analysis.

typical of the Apollo group asteroids. They cannot be the remnants of typical comets but must presumably have been produced at closer distances to the Sun.

We conclude that the majority of dust particles having elliptical orbits detected by Pioneer 8 and 9 space probes show orbital characteristics of Apollo group asteroids which originated from residual nuclei of short-period comets<sup>7</sup>.

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## Generation of magma at lunar impact crater sites

MANY lunar craters have lava flows associated with them. It is widely accepted that, in most cases, the craters are the result of meteoric impact and not volcanic activity but, if so, the occurrence of lavas needs explaining. It is commonly supposed that the lava flows are material that was melted during the impact process itself but this is not acceptable as a general explanation, for two reasons. First, in several cases the ages of the lavas have been found, by crater counting techniques, to be significantly younger than the craters themselves<sup>1</sup> and so they cannot have been formed at the time of impact. Second, some of the lavas show morphology typical of terrestrial lava flows and it is possible to deduce from this that the lava came from sources similar to those of terrestrial volcanoes—that is, lava was supplied at a fairly steady rate over periods of several days and was not simply splash-out from a meteoric impact<sup>2</sup>. It therefore seems that these lava flows are a product of the presence of an impact crater and yet have occurred as normal volcanic effusions long after the formation of the craters.

Terrestrial volcanism is strongly correlated with lithospheric plate margins. This implies that some process occurs at these margins to generate magma since there is no earth-wide magma reservoir. Many mechanisms have been postulated but a likely one is that of frictional or dissipative heating due to relative motion of the plates. Shaw<sup>3</sup> showed that typical lithospheric shear stresses in systems greater than 10 km in extent would probably cause melting in the mantle, as a result of viscous dissipation, in a time of about a million years. Presumably a similar process could operate on the Moon to generate magma.

There is little evidence that there has ever been widespread motion of lithospheric plates on the Moon and it seems unlikely that this could have been the way in which magma was generated. Large scale convective motions within the Moon have been postulated but these are unlikely to penetrate nearer than 300 km to the surface<sup>4,5</sup>. Any magma generated in these motions would not be linked to the formation of impact craters except possibly the largest ones.

There is a further source of motion within the lithosphere which is associated with the formation of impact craters. It is well known that craters become shallower with age and this may be explained by a process of recovery due to viscous creep within the lithosphere. The removal of material from the surface which occurs at the formation of an impact crater produces non-hydrostatic stresses within the lithosphere which are large enough to produce a slow viscous flow. Gash<sup>6</sup> has presented a rheological model of the lithosphere which accounts for the rates of recovery of lunar craters. The model involves

**Table 1** Recovery and melting data

| Crater diameter (km) | Recovery time (Myr) | Time to melting (Myr) |
|----------------------|---------------------|-----------------------|
| 50                   | 21                  | 1.3                   |
| 80                   | 18                  | 0.4                   |
| 130                  | 15                  | 0.15                  |
| 210                  | 11                  | 0.05                  |

viscous flow at stresses greater than a yield value and much slower, though still viscous, flow at weaker stresses. The result is that craters recover relatively rapidly to a depth of about 3 km on the Moon but at a much slower rate when they are less deep. It seems possible that enough heat will be generated during the period of rapid recovery to produce magma beneath a crater. This would explain not only the production of lava at crater sites but also the time interval between the formation of a crater and the lava. Pike<sup>7</sup> discussed several ways in which lava might be generated beneath craters but found no satisfactory explanation; he preferred a process dependent on the recovery of craters to a state of isostasy. The process described here is of this kind.

Preliminary calculations have been made to assess the viability of recovery-induced melting. Gruntfest<sup>8</sup> analysed the phenomenon of dissipative heating in the presence of a temperature dependent viscosity and Shaw<sup>2</sup> applied the results to geophysical situations. Gruntfest<sup>8</sup> showed that the effects of dissipation could be described by a parameter  $G$  which is the ratio of the rate of production of heat due to dissipation to the rate of loss of heat due to thermal conduction. For values of  $G$  less than a critical value of 0.88 in a plane shear situation the effect of dissipation is to raise the temperature slightly. After a characteristic thermal relaxation time the temperature remains steady. For values of  $G$  greater than the critical value a steady state is not achieved; eventually the temperature rises rapidly. For  $G \gtrsim 10$  the system is effectively adiabatic and the temperature theoretically becomes infinite in a finite time. In practice the increasing temperature brings about a change of phase with the result that heat is lost more rapidly, thus braking the process.

$G$  was defined as

$$(\beta\tau^2 l^2)/\lambda\eta_0$$

where  $\tau$  is the shear stress,  $\beta$  the temperature coefficient of viscosity,  $\eta_0$  the viscosity at the initial temperature  $T_0$ ,  $\lambda$  the thermal conductivity and  $l$  the path length for thermal conduction. In the case of the recovery of lunar craters this definition needs to be modified to allow for the non-Newtonian properties of the flow. The rate of dissipation is equal to the stress multiplied by the velocity gradient,  $v$ . For the lunar lithosphere it is assumed, following Gash, that

$$\tau - \tau_y = \eta v$$

where  $\tau_y$  is the yield stress and  $\eta$  the viscosity so that the rate of dissipation equals

$$\tau(\tau - \tau_y)/\eta$$

This falls to zero as the stress reduces to the yield value—that is when the rapid recovery phase ends.  $G$  may be redefined, for this situation, as

$$G = \beta\tau(\tau - \tau_y)l^2/\lambda\eta_0$$

The stress distribution beneath craters is not well known. Jeffreys<sup>9</sup> concluded that a stress of from one-half to two-thirds the surface load would exist initially beneath an excess load. For the case of a surface indentation it seems that some concentration of stress will occur<sup>6</sup> and so it is a reasonable approximation that the maximum non-hydrostatic stress beneath a crater will be equal to the maximum surface load which is  $(g\rho d)$  where  $d$  is depth of the crater and  $\rho$  the density of the lithosphere. The depth changes in time, due to the recovery process, and the change may be expressed by

$$d = d_0^{-\alpha} \exp(-\alpha T)$$

where

$$\alpha = g\rho D_0/4\pi F\eta$$

$D_0$  is the initial diameter of the crater, and  $F$  is an empirical factor introduced by Gash<sup>6</sup>. The situation is further complicated by the fact that the rate of recovery—and hence the rate at which the stress changes—is viscosity dependent.

Apart from the steady decrease in stress there are other difficulties in evaluating  $G$  for the case of lunar craters. As the temperature rises the rheology of the lithosphere will change: the yield stress will decrease and the temperature coefficient of viscosity is unlikely to remain constant. Gruntfest<sup>8</sup> assumed a variation of the type

$$\eta = \eta_0 \exp(-\beta(T - T_0))$$

For a solid, the creep viscosity is more likely to follow<sup>4</sup>

$$\eta = \eta_0 T e^{E/kT},$$

where  $E$  is the activation energy for viscosity and  $k$  is Boltzmann's constant. This means that

$$\beta \sim -E/kT^2$$

and therefore varies with temperature. Tozer<sup>4</sup> predicts that  $E/k$  for the lunar lithosphere will be about 25,000 K so that  $\beta$  will range from about 0.02 just below the melting point to almost 0.3 at 300 K. Finally, the value of  $l$  is not well defined. For a crater, the depth at which maximum stress occurs is probably near to 25% of the crater diameter<sup>9</sup>. Therefore, the conduction path to the surface is probably the dominant one and  $l$  should be put equal to  $D_0/4$  although the region in which heating occurs may have a horizontal extent which is greater than this.

It follows that, for a crater,

$$G = \beta\tau(\tau - \tau_y) D_0^2/16\lambda\eta_0$$

Following Gash<sup>6</sup> it is assumed that  $\eta_0 = 4 \times 10^{21}$  Pa s and  $\tau_y = 1.7 \times 10^7$  N m<sup>-2</sup>. Before any rise in temperature  $\beta$  will be about 0.25 K<sup>-1</sup> and  $\lambda$  will be approximately 2 W mK<sup>-1</sup>. With these values  $G$  will be equal to its critical value at the start of the recovery process for a crater about 35 km in diameter. This is about twice the diameter of the smallest craters subject to rapid recovery<sup>6,7</sup>. This suggests that the recovery of a crater of this size may eventually produce high temperatures. It seems that the recovery of craters which are not much larger would almost certainly give rise to melting but it must be remembered that as the temperature rises both the stress and the rate of change of viscosity with the temperature decrease and so tend to reduce the rate of temperature rise. The heating will cease when the rapid recovery phase is completed and so a further requirement for melting to be achieved is that high temperatures must be produced before rapid recovery is complete.

If it is assumed that  $G$  is large enough for the process to be adiabatic then the time,  $t_{ad}$ , taken for the temperature to become very large is

$$t_{ad} = \rho c \eta_0 / [\beta\tau(\tau - \tau_y)]$$

where  $c$  is the specific heat of the lunar material. The ratio  $M$  of recovery time to the time required to achieve melting reduces to

$$M = (4\pi F g \beta / c) (d_0/D_0)^2 (D_0 - D_s) \ln(D_0/D_s)$$

where  $D_s$  is the diameter of the smallest crater subject to rapid recovery and is somewhat less than 20 km on the Moon. The ratio  $M$  must be large if melting is to occur. For a 35-km diameter crater  $M$  is about 7 and so high temperatures will be produced long before recovery is complete. Some examples of characteristic recovery times for the rapid recovery phase and times needed to achieve melting are given in Table 1.

It is likely that these melting times are underestimates because of the tendency for the rate of heating to decrease as the



temperature rises and as recovery proceeds. It seems clear from these preliminary calculations that viscous dissipative heating could have been of major importance in the recovery of larger lunar craters. More elaborate computerised calculations may help to reveal more details of the process when more of the complexities of the situation are accounted for.

The arguments given here suggest that, for lunar craters larger than a certain size—not much greater than the minimum size of craters which undergo rapid recovery—the process of recovery gives rise to a great increase in temperature in a region beneath the crater and probably causes melting. It is hard to estimate how much melting might occur because the extent of the heated region is not known. The calculations of Gruntfest<sup>8</sup> showed that the temperature of a sheared slab does not increase equally at all points. Although the material is initially homogeneous its response to shear stress is heterogeneous and melting first occurs in a very narrow region. Furthermore, experimental work with liquids which show a marked change in viscosity at some yield stress has shown that the regions in which the lower viscosity is displayed are very narrow when the stress is of the order of the yield stress<sup>9</sup>. These regions appear as shear planes along which the strain rate is high. This evidence suggests that melting will occur in narrow zones along shear planes in the lunar lithosphere. The temperature will continue to rise and the amount of melting will increase until the system is disrupted—probably by an effusion of magma to the surface. This process may be repeated periodically. It seems that the initial melting may take up to  $10^7$  yr for the smaller craters but perhaps as little as  $10^4$  yr for the largest craters.

The depth at which melting occurs is likely to be related to crater diameter and is probably of the order of 25% of the diameter. For most craters the melting region will be within the cool lithosphere but beneath the largest craters dissipation of energy will occur in deeper material which is initially much warmer and of more basic composition than the outmost layers of the Moon. Melting will be achieved in shorter times than those given in Table 1 when the material is warmer, because of the reduced viscosity.  $G$  will remain supercritical for relatively longer in the larger systems with lower viscosity and so many more episodes of melting may occur before recovery is complete in larger craters. It would be expected that the composition of lavas would be dependent on crater diameter and this is what is generally observed. Lighter, more acidic lavas are associated with smaller craters whose sources are near the surface and dense basaltic lavas from deep in the Moon are found in the largest craters and the maria. For example, the lavas associated with the crater Tycho apparently have higher yield stresses and hence are probably more acidic than those of Mare Imbrium<sup>3</sup>.

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## Dependence of seismic mean residuals on distance, azimuth and time

MOST studies on the changes of seismic velocity which precede earthquakes (see, for example, refs 1–5) are based on data from highly sensitive, but local, seismic networks. Large earthquakes very rarely occur in the neighbourhoods of these networks, so to study velocity changes associated with earthquakes of large magnitude, it is better to use a worldwide network of stations such as those reporting to the International Seismological Centre (ISC) in Edinburgh. Studies of this kind, on the teleseismic residuals over an eight-year period at Matsushiro, have been reported by Wyss and Holcomb<sup>6</sup>. Their results show a clear

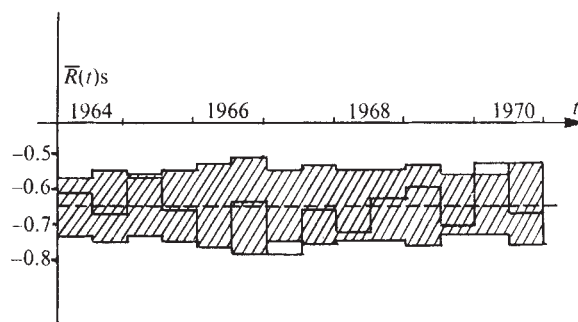


Fig. 1 The continuous line shows the mean residuals for the fourteen half-yearly periods covered by this study. — — —, Overall mean residual. Hatched area denotes the 95% confidence limits for each half-yearly period, centred on the overall mean.

jump in the mean P-wave residuals in 1963, 2.5 yr before the beginning of a large earthquake swarm in the vicinity of the seismic station. Since the variation is very close to the level of statistical significance, I have made a study of similar anomalies at other stations close to the epicentres of strong earthquakes. The period covered by the data, which have been taken directly from the ISC Bulletins, is 1964–70.

Each six-month period was taken separately and all residuals under 5 s for first arrivals of P waves were used. These were divided into groups by azimuth ( $90^\circ$  sections) and by distance ( $0-10^\circ$ ,  $10-20^\circ$ ,  $20-40^\circ$ ,  $40-60^\circ$  and more than  $60^\circ$ ), and the mean was calculated for each group (and for each six-month period and for every listed station).

Mean residuals were first calculated for seismological stations in aseismic areas in order to test whether the averaging procedure really cancels the influence of factors like differences in source mechanism, propagation path and so on. (The spatial pattern of earthquakes in different periods of time may, for instance, have a strong effect on the time variation of the mean residuals.)

As an example, the mean residuals for the Uppsala station are shown in Fig. 1. The histogram summarises the results for the 14 half-year periods. The residuals lie within  $\pm 0.14$  s of the overall mean value of  $-0.65$  s, thus showing good stability. In two cases the observations lie outside the confidence limits, so there still remains the possibility of slight non-random variations in the mean residuals, but undoubtedly the averaging procedure is reasonably effective in suppressing irrelevant effects.

The result of  $-0.65$  s for the overall mean residual, with a standard error of 0.01 s, was calculated from 8,553 residuals. The negative value seems to correlate with the known tendency of the Uppsala station to assign high values to the magnitudes of some seismic events. Early arrivals (negative residuals) correspond to high velocities which are normally associated with low attenuation (high  $Q$ ) and, therefore, large magnitudes. Many other Scandinavian stations have negative mean residuals,

**Table 1** Dependence of mean residuals at the Uppsala station on distance and azimuth

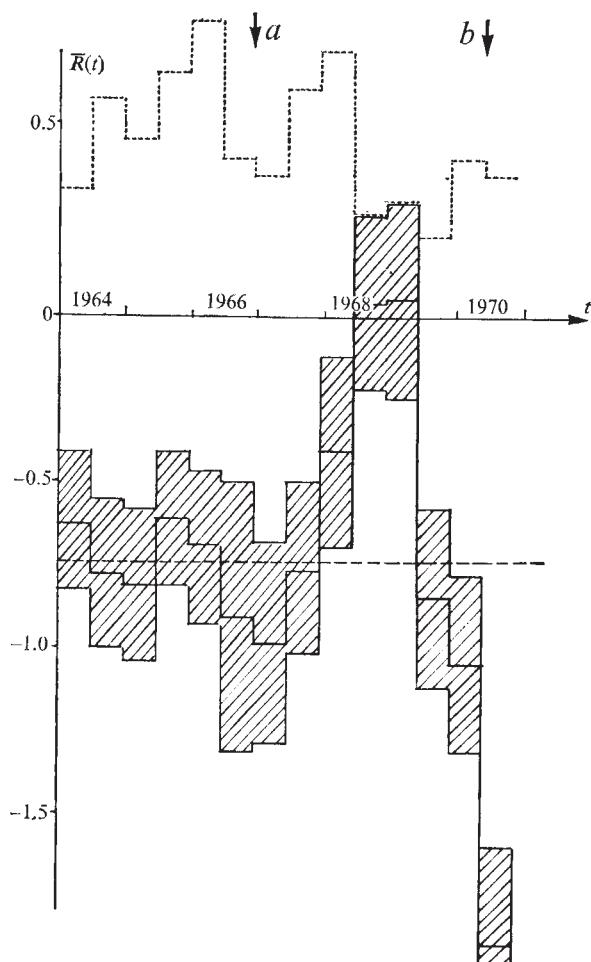
| Azimuth | 0°–10°            | 10°–20°           | Distance<br>20°–40° | 40°–60°           | >60°                | Total               |
|---------|-------------------|-------------------|---------------------|-------------------|---------------------|---------------------|
| North   | 0.57±0.37<br>35   | –0.50±0.17<br>015 | –0.91±0.05<br>1,162 | –0.64±0.04<br>899 | –0.65±0.02<br>5,630 | –0.68±0.01<br>7,891 |
| East    | 0.30±0.23<br>34   | –1.30±0.42<br>19  | 0.00±0.22<br>47     | 0<br>0            | 0<br>0              | –0.14±0.16<br>100   |
| South   | –0.84±0.21<br>42  | –1.94±0.34<br>34  | 0.24±0.63<br>16     | –0.35±0.16<br>0   | 0<br>0              | –1.06±0.21<br>92    |
| West    | 0.07±0.37<br>40   | –1.13±0.97<br>6   | –0.94±0.08<br>74    | –0.63±0.04<br>22  | 0<br>0              | –0.58±0.13<br>142   |
| Total   | –0.02±0.16<br>151 | –0.80±0.14<br>224 | –0.87±0.04<br>1,299 | –0.63±0.04<br>921 | –0.65±0.02<br>5,630 | –0.65±0.01<br>8,553 |

The standard error is included with each evaluation and the number of residuals used is shown.

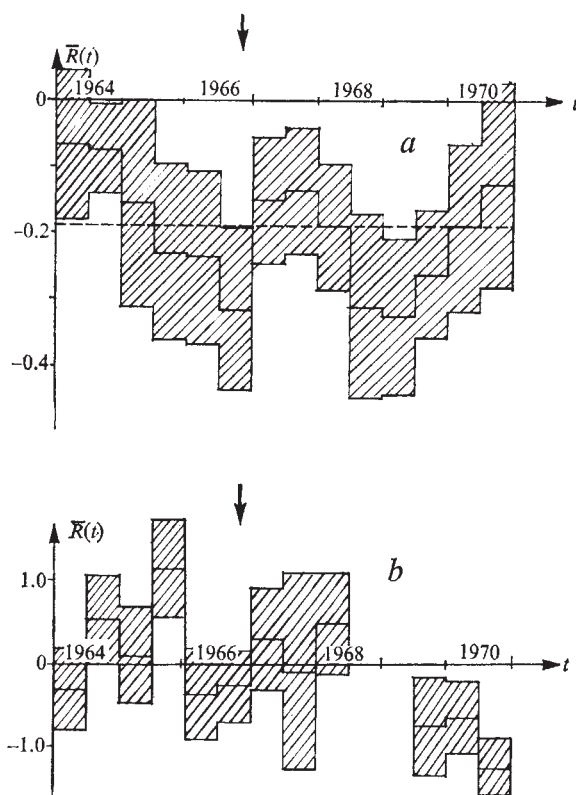
the magnitudes of which increase towards the centre of the Scandinavian peninsula. In North America, apart from a cluster of stations in central California with positive residuals, stations have mainly negative residuals. This too, seems to correlate with the distribution of magnitude anomalies for North American stations (A. Douglas, personal communication).

Results from many other stations in aseismic areas show the same stability. Some, however, reveal statistically significant changes in time over the period 1964–70. This would be expected in cases where changes of instrument or method of detection of first arrivals have taken place. At some stations there is a strong dependence of residuals on distance and azimuth (see, for example, Table 1).

I now consider four sets of residuals at stations situated in the vicinity of large earthquakes which occurred in the period 1964–70. The first set is from the Nana and Huancayo stations, which are near the epicentres of the 1966 and 1970 Peruvian earthquakes. Figure 2 shows the variation of the residuals with time.



**Fig. 2** Mean residuals at station Nana for the fourteen half-yearly periods with the 95% confidence limits denoted by hatching. — — —, Mean residuals for station Huancayo. The arrows show the times of onset of the 1966 and 1970 Peruvian earthquakes; *a*,  $M = 7.75-8$ , October 1966,  $\Delta = 2.2^\circ$ ; *b*,  $M = 7.6$ , May 1970,  $\Delta = 3.4^\circ$ .



**Fig. 3** Mean residuals with 95% confidence limits for Antofagasta (*a*) and Copiapa (*b*). The arrows show the time of onset of the December 1966 Chilean earthquake; *a*,  $M = 7.75-8$ ; *b*,  $M = 7.75-8$ .

A statistically significant reduction in seismic velocities occurred at Nana two years before the latter event. An analogous reduction, smaller in size and occurring slightly earlier, appears for Huancayo which is a little further from the epicentre (shown without confidence limits as a dotted line in Fig. 2). On the other hand, the first earthquake is preceded by a jump in the residuals at Huancayo station only; variations at Nana before 1968 are statistically insignificant.

A second example is the Chilean earthquake of December 1966. There were two stations close to the epicentre ( $\Delta \sim 2^\circ$ ) but on almost opposite sides, Antofagasta and Copiapa. They show significant changes in mean residuals before the earthquake (see Fig. 3*a* and *b*), but they have a rather complicated structure. The pattern of a drop in velocity one or two years before the earthquake is, however, repeated at each station.



Figure 4 shows the time variation of the residuals at the Przevalsk station,  $0.23^\circ$  from the epicentre of a magnitude 6.8 earthquake in 1970. They follow the pattern of the Nana residuals very closely. Unfortunately, reports from the station to the ISC ceased for a 1.5-yr-period preceding the event. Significant changes in level were reported by Bullanzhe, Atrushkevich<sup>7</sup> and others in Alma Ata city, which is close to Taldar, following the earthquake.

The final example (Fig. 5) is taken from data at the Makhachkala station, situated  $0.24^\circ$  from the epicentre of a strong Dagestan earthquake in 1970. Because of the small number of events reported by this station, the statistics are rather poor. The pattern of variations of the mean is, however, quite similar to those of the preceding examples.

In conclusion, mean residuals of P-wave arrivals ( $R$ ) show statistically significant time variations at seismic stations which are close to future earthquakes. The variations have a rather

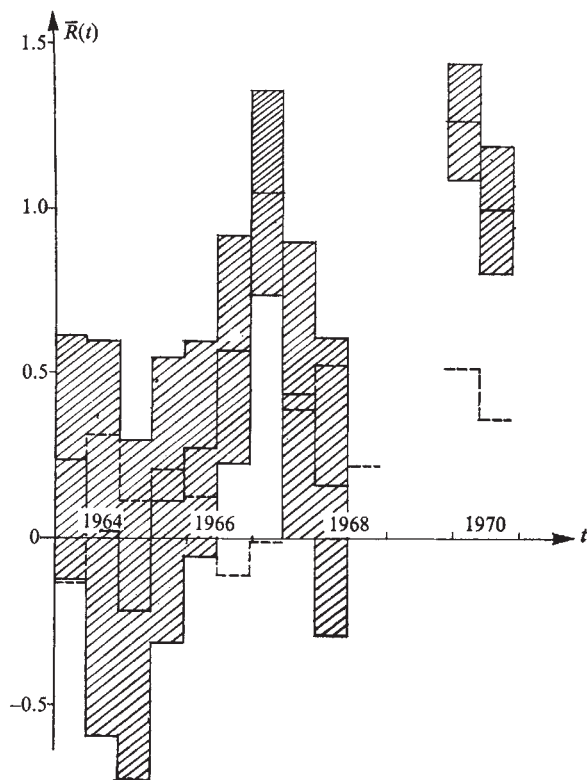


Fig. 4 Mean residuals with 95% confidence limits for Przevalsk. — — —, Mean residuals for Taldar, a station situated five times further away. The arrow shows the time of onset of the June 1970 earthquake;  $M = 6.8$ .

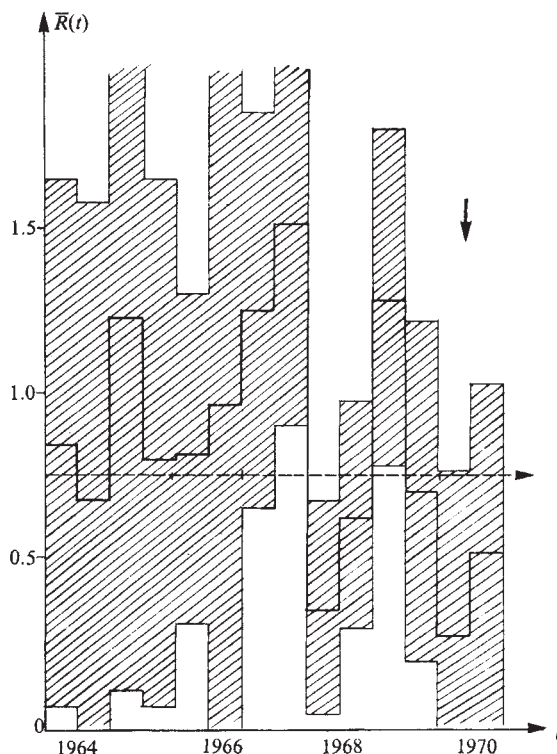


Fig. 5 Mean residuals with 95% confidence limits for Makhachkala. The arrow shows the time of onset of the May 1970 Dagestan earthquake;  $M = 6.8$ .

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complicated structure but the main general feature is a jump in value before the earthquake. This corresponds to a decrease in velocity and is in agreement with the predictions of the dilatancy hypothesis. The distance at which variations of  $R$  could be detected is a few times larger than the sizes of the source as determined by the aftershocks zone and by level measurements.  $R$  depends strongly on the distance and azimuth of the source. It is correlated with magnitude anomalies in a way which confirms the physical interpretation: lower attenuation corresponds to higher seismic velocities.

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## Seismic slip rates in the Mediterranean and Middle East

VARIOUS plate tectonic models have been suggested for the Mediterranean and Middle East<sup>1-3</sup>. These differ in detail, but all require several smaller plates to account for the complicated seismicity observed, a simple interaction between the African, Arabian and Eurasian plates being insufficient for this purpose. It has been pointed out<sup>4,5</sup> that recent developments in the theory of earthquake mechanism, most notably the concept of seismic moment<sup>6</sup>, enable determinations of the extent to which interactions between plates affect the rates of seismicity along their boundaries. Use of earthquake catalogues



Fig. 1 Events for which seismic moment has been determined from Rayleigh wave spectral amplitudes.

for the first half of this century<sup>7,8</sup> may be combined with a magnitude-moment relation to extend the relatively short time interval for which reliable measurements of moment can be made (the last 10–15 yr), though such studies are necessarily subject to considerable error.

In spite of the large inaccuracies involved, a comparison<sup>5</sup> of relative motions between the major plates determined from seismicity over the last 70 yr with those calculated on the basis of plate tectonic theory from, for example, seafloor spreading evidence, indicated considerable agreement between the two. A notable exception to this was the western part of the Alpine Belt, where the calculated slip rate was considerably greater than that evidenced by the seismicity. In the present study accurate determinations of seismic moment have been made for all the larger ( $M_s > 5.5$ ) earthquakes in this region for the period 1963–70. These results have been used to construct a magnitude-moment relation for estimation of seismic slip between 1910 and 1970, for comparison with the rates of motion predicted by a plate tectonic model<sup>1</sup> for the region.

The latter extends from the Azores to eastern Iran (60E). The fault-plane solutions determined<sup>1</sup> were used to compute the theoretical Rayleigh wave spectra for the 80 events (Fig. 1) large enough to have digitisable surface waves. The procedure adopted in the reduction of the surface-wave data is essentially that of previous workers<sup>6,9,10</sup>. As the region studied is structurally very complex, considerable crustal and upper mantle variations will unpredictably affect the shorter periods, so observed and theoretical spectra are compared only in the period range 40–80 s.

For three of the largest events, seismic moment has already been determined<sup>11,12</sup> and these results are used here. Some of the larger shallow earthquakes in Turkey and Iran occurring before 1963 produced appreciable and well-documented surface displacements<sup>13,14</sup> from which moment may be estimated. In Fig. 2 all the moment values obtained are plotted against the corresponding surface-wave magnitude  $M_s$ . Shown on the same figure (dashed lines) are two magnitude-moment relations (B<sup>15,16</sup> and A<sup>17,18</sup>) calculated on the basis of scaling laws of the earthquake source. In the magnitude range  $6 \leq M_s \leq 7.5$  the relation used here is given by the solid line (C): it gives generally higher moment values than either A or B. For  $M_s > 7.5$  there is considerable disagreement between B and A as to the nature of the magnitude-moment relation: the data presented here do not convincingly favour either. Fortunately, all but one (Azores–Gibraltar region, November 25, 1941) of the higher magnitude events ( $M_s > 7.5$ ) in the area during 1910–60 were associated with surface displacements from which we may estimate moment (Fig. 2).

Relation C now permits calculation of the relative motion occurring along the major fault zones in the region considered. On the basis of the plates suggested<sup>1</sup>, the Mediterranean and Middle East has been divided into 14 plate boundary 'segments' (Fig. 3 and Table 1). A search has been made through earthquake catalogues<sup>7,19</sup> for the area and each event of  $M_s \geq 6$  assigned, wherever possible, to one of these boundary segments. For each event moment has been estimated and the moment sum for each segment calculated. We consider only the 60 yr

Table 1 Theoretical (th) and observed (obs) moment rates in units of  $10^{24}$  dyne cm yr<sup>-1</sup>

| No. | Segment identification | L(km) | Faulting type* | V*(cm yr <sup>-1</sup> ) | Plates involved | $M_o^{obs}$ |                      | N      | % $M_o$ | $M_o^{th}$ | $M_o^{th}/M_o^{obs}$ 1910–70 |
|-----|------------------------|-------|----------------|--------------------------|-----------------|-------------|----------------------|--------|---------|------------|------------------------------|
|     |                        |       |                |                          |                 | 1963–70     | 1910–70              |        |         |            |                              |
| 1   | Azores to 18W          | 900   | S              | 0.9                      | AF-EU           | 1.2         | 340 (B)<br>16600 (A) | 4<br>4 | 97      | 146<br>146 | 0.43(B)<br>0.0088(A)         |
| 2   | 18W to Gibraltar       | 1,300 | T              | 1.1                      | AF-EU           | 770         | 110                  | 6      | 86      | 515        | 4.7                          |
| 3   | Gibraltar–Tunis        | 1,400 | T              | 1.5                      | AF-EU           | 4.7         | 7.5                  | 7      | 37      | 422        | 56                           |
| 4   | Sicily/Italy/Alps      | 2,400 | M              | 2.0                      | AF-EU           | 13.2        | 105                  | 16     | 34      | 1,270      | 12                           |
| 5   | Yugoslavia/Albania     | 1,000 | T              | 2.0                      | AF-EU           | 26.8        | 6.9                  | 10     | 31      | 528        | 77                           |
| 6   | Cretan arc             | 1,100 | T              | 3.7                      | AF-AE           | 13.2        | 88                   | 27     | 41      | 1,370      | 16                           |
| 7   | Northern Greece        | 500   | N/S            | 2.0                      | EU-AE           | 122         | 98                   | 45     | 10      | 188        | 1.9                          |
| 8   | Western Turkey         | 400   | N              | 3.0                      | AE-TU           | 86.3        | 87                   | 24     | 41      | 241        | 2.8                          |
| 9   | Anatolian fault        | 1,100 | S              | 3.2                      | TU-EU           | 222         | 423                  | 23     | 46      | 352        | 0.83                         |
| 10  | South Turkey           | 900   | T/S            | 3.5                      | AF-TU           | —           | 1.9                  | 3      | 47      | 865        | 455                          |
| 11  | Jordan/Syria           | 900   | N/S            | 0.5                      | AR-AF           | —           | 1.0                  | 2      | 61      | 45         | 45                           |
| 12  | Border zone            | 600   | S              | 4.2                      | AR-TU           | —           | 3.2                  | 2      | 88      | 250        | 78                           |
| 13  | E. Turkey/Caucasus     | 600   | T              | 3.8                      | AR-EU           | 12.1        | 9.4                  | 10     | 17      | 750        | 80                           |
| 14  | Iran                   | 2,000 | T              | 4.5                      | AR-EU           | 25.5        | 183                  | 46     | 15      | 2,950      | 16                           |

Segment numbers and plate names from Fig. 1. The predominant type of faulting is designated strike slip (S), thrust (T), normal (N) or mixed (M). N is the number of events for which moment has been summed and the next column gives the % of total moment contributed by the largest event.

\* From ref. 1.



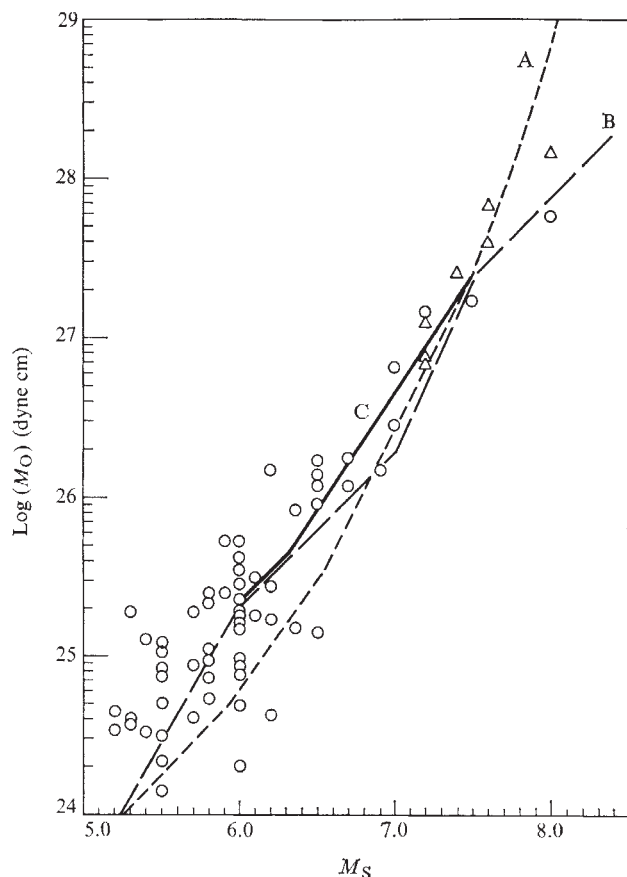


Fig. 2 Seismic moment as a function of magnitude for the events studied here (O) and for those for which moment could be estimated from surface faulting evidence ( $\Delta$ ). Also shown are the magnitude-moment relations A, B and C discussed in the text.

period 1910–70, mainly because before 1910 magnitude determinations were rarely instrumental and therefore likely to be unreliable. In Table 1 the moment sums for each segment over 1910–70 are given, together with those for 1964–70 during which moment measurements are reliable. Each has been reduced to a yearly rate and, as expected, these differ considerably, reflecting secular variations in seismicity.

For segment 1 two values are given, corresponding to a conversion of the magnitude of the large earthquake already mentioned to moment through relations B and A. The difference between the prediction of these models is very large: at  $M_s = 8.25$ ,  $M_0(A)/M_0(B) = 500$ . In Table 1 the number of events of  $M_s \geq 6$  ( $N$ ) in each segment is given, together with the percentage of total moment contributed by the largest event; where this exceeds 50% the moment rate may well be a poor reflection of one based on longer term seismicity.

The rates calculated from the plate motions suggested<sup>1</sup> are also given in Table 1. The relative yearly rates of motion ( $v$ ) between these plates are obtained from the velocity vectors given and each is an average over the length  $L$  of the plate boundary segment. The depth  $D$ , to which faulting takes place is taken as 30 km except in mountainous areas (segments 5, 13, 14) where, by analogy with results for the Caucasus<sup>20</sup>, it is taken as 50 km. Seismic moment is defined as  $M_0 = \mu u A$ , the product of the shear modulus  $\mu$ , (taken as  $3.3 \times 10^{11}$  dyne  $\text{cm}^{-2}$ ) the area of the faulting boundary  $A (=LD)$  and the mean relative displacement  $u$ . For a region of strike slip faulting the yearly moment rate is then  $M_0 \text{ yr}^{-1} = \mu v L D$ . For areas of thrust or normal faulting, involving crustal shortening or extension,  $M_0 \text{ yr}^{-1} = 2\mu v L D / \sin \delta$  where  $\delta = 45^\circ$  is the average dip of the planes on which faulting takes place.

In the last column the ratio of the theoretical moment rate to the observed (equivalent to the ratio of slip rates) is given for the period 1910–70. For all but four of the segments, the percentage of moment contributed by the largest event is less than 50%, and so a ratio close to unity might be expected, on the basis of that observed elsewhere in the world<sup>9</sup>. This seems, however, to be the case only for segments 7, 8, and 9, and an alarming feature is that for no individual plate is there even reasonable agreement along all its boundaries. The best-known plate interactions, those of Africa–Eurasia and Arabia–Eurasia, show particularly poor agreement. It can reasonably be argued that observed and theoretical slip rates should be in closer agreement when the latter are large, as the seismicity should be higher and a shorter period of time sufficient. A comparison of the ratio of theoretical to observed slip rates with predicted slip rates ( $v$ ) reveals that this is not the case. A curious feature is that the motions of the smaller plates such as the Aegean and Turkish, are, at least along some of their boundaries, close to those predicted.

The seismic slip rates obtained in this study are not only generally much less than those predicted by what seems to be a quite plausible model for the tectonics of this region, but are also far lower than those observed elsewhere. For the large magnitude earthquakes the moments can, with one exception, be estimated from surface faulting. In the magnitude range  $6 \leq M_s \leq 7.5$  the magnitude-moment relation derived predicts higher moment values than those given by relations A and B, and so these are likely, if anything, to be too high. The magnitudes themselves are unlikely to be systematically low and would need to be so by at least one unit to produce the discrepancies observed. Errors in the values chosen for the vertical extent of faulting and the shear modulus are unlikely to be more than 50%, and can hardly cause the considerable disagreement found.

The latest model<sup>1</sup> of the tectonics of this region is therefore not capable of verification by the methods described in this paper, on the basis of either present or past seismicity. No individual slip rate obtained is considered reliable enough to constitute a contradiction of the model, and in particular some of the boundaries of the minor plates are almost as active as predicted. If the earthquakes in this area are considered to result solely from an interaction between the Eurasian, African and Arabian plates, the observed seismicity rate is still an order of magnitude less than expected. None of the results obtained here contradict the existence of the smaller plates, which are in any event required to produce the complicated pattern of seismicity.

There are two possible causes for these large discrepancies. The first, and most obvious, is that 70 yr is not a long enough time for the seismic slip rates to agree with those calculated from the plate theory. If this were so, then one might expect, on the basis of the agreement found elsewhere<sup>9</sup>, that the ratios of observed to predicted slip rates would vary about unity. This is

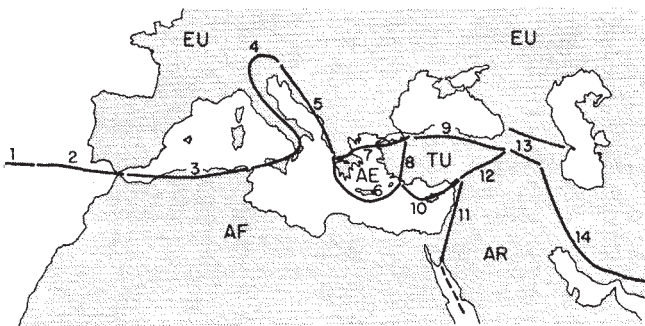


Fig. 3 Plate boundary segments to which events in this area were assigned. The plates shown are the Eurasian (EU), African (AF), Arabian (AR), Aegean (AE) and Turkish (TU).

not the case, this ratio being generally low. The most plausible explanation of the considerable discrepancies observed here is that a major proportion of the deformation in this area takes place in viscoelastic processes such as creep. This should be verifiable by the installation of strain/creepmeters in the regions where the disagreement between theory and observation is greatest.

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## Submerged platform of marine abrasion around the coasts of south-western Britain

RECENT studies at Aberystwyth have shown that Palaeozoic rocks extend as a gently sloping platform (below a cover of sediment or drift) 16 km seawards of the coast of south-western Wales, up to 18 km to the south of the coast of Ireland, and some 15 km north-west of Cornwall. Sparker traverses<sup>1</sup> show this platform to be, in general, smoothly planed with its outer margin descending to at least 80 m. Former islands, now submerged, occur; as, for example, in St. Bride's Bay and off Tuskar Rock. Westwards of the Camel estuary the platform continues unbroken across the fault which throws Cretaceous rocks against the Palaeozoic. Shorewards no definite submerged cliff line has so far been traced and in some cases at least, the cliff line may be coincident with the outer edge of the modern coastal platform, or even the modern cliff<sup>2</sup>.

A platform of this width, cut in resistant Palaeozoic rocks, can hardly have been cut during the successive low sealevels of the Pleistocene, which occupied in total only a small proportion of

Pleistocene time. The interglacial '10 foot platform' recognised in southern and western Wales<sup>3</sup>, Devon<sup>4</sup> and southern Ireland<sup>5</sup> forms only an insignificant nick in the coastal cliffs. Moreover, this platform, probably of Cromerian age<sup>6,7</sup>, runs into already formed estuaries and bays<sup>8</sup>, and was thus preceded by a period of lower sealevel. The St Erth Beds of late Pliocene age similarly occur<sup>9,10</sup> on the side of a pre-existing valley. The broad submerged platform of marine abrasion is, therefore older than late Pliocene.

This gives the *terminus ad quem*, what of the *terminus a quo*? The Lenham Beds of south-eastern England occur as residual deposits on the tops of hills and must precede this period of lower sealevel and prolonged erosion. It is inconceivable that deposits coeval with the Lenham Beds would not have been found in the valleys of the Weald had those valleys been in existence and submerged by the Lenham sea. Equivalents of the Lenham Beds<sup>11</sup> found in borings in the Antwerp Kampen contain Upper Miocene foraminifera, confirming an opinion previously expressed by Voorthuysen<sup>12</sup>. The cutting of the submerged platform therefore occurred between Upper Miocene and late Pliocene times.

Evidence seems to favour an end Miocene or early Pliocene date. Gignoux<sup>13</sup> has remarked on the regression of latest Miocene time, which affected western Europe. The late Miocene (Messinian) desiccation of the Mediterranean postulated by Ruggieri<sup>14</sup> has been confirmed<sup>15</sup>. This event occupied 2 Myr according to the time scale of Berggren<sup>16</sup>. The platform must have been trimmed and trimmed again during the successive regressions and transgressions of the Pleistocene but its formation occurred much earlier. Likewise, submerged river valleys crossing the shelf<sup>17</sup> are likely to be very old, having merely been scoured out during the last regression of the sea and refilled by Flandrian deposits.

Not the least surprising feature, if this hypothesis is true, is the remarkable stability of western Britain during this long period of time, contrasting so markedly with the activity of the marginal faults during the Oligocene and Miocene. The high-level platforms of Wales, Cornwall and Ireland must necessarily have been formed in Oligocene and Miocene times, the submerged platform being simply the last of a series of erosional features occasioned by prolonged stillstands of the sea.

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## 'Spectacle' array of $^{210}\text{Po}$ halo radiocentres in biotite: a nuclear geophysical enigma

POLONIUM radiohaloes occur widely and not infrequently (total about  $10^{15}$ – $10^{20}$ ) in Precambrian rocks but their existence has so far defied satisfactory explanation based on accepted nucleocosmogeological theories<sup>1</sup>. Do Po haloes imply that unknown processes were operative during the formative period of the Earth? Is it possible that Po haloes in Precambrian rocks represent extinct natural radioactivity<sup>2</sup> and are therefore of cosmological significance? A detailed comparison between an unusual array of Po halo radiocentres and U–Th halo radiocentres is presented here as bearing on the above questions.

Generally, radiohaloes occur in one of several mineralogical contexts<sup>1,3,4</sup>. First, as single haloes around discrete inclusions well isolated from other mineral defects and haloes; second, as single haloes around discrete inclusions lodged in conduits or cleavage cracks; third, as single haloes randomly spaced in clusters (sometimes overlapping); fourth, as vein haloes which formed from a continuous distribution of radioactivity (apparently deposited from hydrothermal solutions) along a conduit; and fifth, as line haloes, which surround, not conduits or cracks, but genuine single inclusions which are long (for example, 25  $\mu\text{m}$ ) compared with their width (perhaps 1  $\mu\text{m}$ ). Large, amorphous, coloured regions without discrete inclusions are not haloes.



Fig. 1 'Spectacle' array of  $^{210}\text{Po}$  haloes in biotite. Halo radius, 18.5  $\mu\text{m}$ .

A striking exception<sup>1</sup> to this classification is the 'spectacle' coloration pattern (Fig. 1), which exhibits two almost circular rings of inclusions joined by a linear array of inclusions. As far as we know this is unlike any group of haloes previously seen. This geometrical arrangement of halo radiocentres, found in a Precambrian biotite from Silver Crater Mine, Faraday Township, Ontario, exhibits true radiohalo characteristics.

First, the coloration is identical to that of normal haloes found about 300  $\mu\text{m}$  away in the same mica specimen. Second, the three-dimensional nature of the halo pattern was demonstrated when the specimen (initially about 50  $\mu\text{m}$  thick) was cleaved; both halves revealed matching 'spectacle' coloration patterns, the only difference being the presence of the inclusion array in one half and its absence in the other half. Third, the radius of the coloration band (18.5  $\mu\text{m}$ ) implied an origin from  $^{210}\text{Po}$   $\alpha$  decay. Mass spectrometric and X-ray fluorescence

methods were used to ascertain whether this was indeed a Po halo array.

Before applying these techniques to the 'spectacle' halo, we established that ion-microprobe mass analyses and scanning electron microscope X-ray fluorescence (SEM-XRF) studies of 'normal' or 'standard' halo radiocentres (those formed from both U and Th  $\alpha$  decay) yielded data consistent with the visual means of identification. Several U–Th haloes (see, for example, photo insert, Fig. 2) found in a Precambrian pegmatitic mica from Rossi, New York, were analysed by X-ray and ion-probe techniques. Several U–Th halo radiocentres were chosen which contained only U, Th and Pb in any significant abundance, thereby virtually eliminating any molecular ion interference in the Pb–Th–U region ( $m/e = 204$ – $238$ ) in the ion probe.

That the mica matrix<sup>5</sup> yielded insignificant molecular ion currents in the region  $m/e = 160$ – $320$  is evident from the data in the lower portion of Fig. 2. In contrast, the recorded spectra of a U–Th inclusion (upper left portion of Fig. 2) revealed a significant number of ion counts accumulated in 12 passes of the regions  $m/e = 204$ – $209$  and (with a different scale)  $m/e = 232$ – $240$ . Total ion counts are tabulated just above the two spectra. The scans on the Pb–Bi region ( $m/e = 204$ – $209$ ) lasted for several minutes and were taken before the scans (equal time) on the U–Th region.

Exact  $^{206}\text{Pb}/^{238}\text{U}$  and  $^{208}\text{Pb}/^{232}\text{Th}$  ratios are not obtainable from the ion count data in Fig. 2 because variable U and Th concentrations were observed as the ion probe beam sputtered away the inclusion; accurate ratios could be obtained by simultaneously accumulating counts in the region 204–238 provided that the greater secondary ion yield of U and Th as compared with Pb is taken into account. On the other hand, the separate Pb and U isotope ratios are meaningful. Note, for example, that after subtraction of background counts at  $m/e = 240$  from the total counts at  $m/e = 235$  and 238, the 235/238 value (0.76) satisfactorily approximates (considering the relatively small number of counts collected) the natural U isotopic ratio,  $^{235}\text{U}/^{238}\text{U} = 0.72$ . The absence of a peak at 204 shows there is little or no common lead in the inclusion and therefore, that the 206/207 ratio is that of  $^{206}\text{Pb}/^{207}\text{Pb}$  as derived from *in situ* U decay.

Also shown in Fig. 2 are the SEM-XRF spectra of the mica matrix and the U–Th halo radiocentre, both of which correlate well (with the exception of the low Z and low abundance elements in the former) with the respective ion-probe spectra. Only U, Th and Pb are exclusively in the inclusion.

The ion-microprobe mass spectrum of the mica matrix surrounding the 'spectacle' halo was nearly identical to the mica spectrum shown in Fig. 2 and is not repeated in Fig. 3. Figure 3 (top centre) shows the portion  $m/e = 160$ – $264$  of the ion-microprobe spectrum (vertical log scale) of several of the inclusions. Also shown is the actual ion-probe trace of the important region from  $m/e = 204$ – $210$  using a linear vertical scale and an expanded horizontal scale. There is no significant ion current above  $m/e = 209$ ; that is, no significant ion signals were detected at any of the prominent U and Th peaks: 238( $\text{U}^+$ ), 254( $\text{UO}^+$ ), 232( $\text{Th}^+$ ) and 248( $\text{ThO}^+$ ). No  $m/e = 204$  was detected above background (1 c.p.s.), and the 206/207 mass ratio was  $\approx 20$  (206 signal  $\approx 2,000$  c.p.s.).

Figure 3 also shows SEM-XRF spectra of the surrounding mica and of one of the Po halo radiocentres. Lead is the only element detectable in this radiocentre exclusive of the mica; some adjacent radiocentres revealed Bi as well. The use of two different instruments, and longer counting times, account for the slightly different X-ray spectra in Figs 2 and 3. The excellent resolution of the SEM showed the Pb-rich areas to coincide exactly with the Po halo radiocentres which are visible both in ordinary transmitted (Fig 1) and reflected light microscopy. Regions as close as 1  $\mu\text{m}$  to the radiocentres showed virtually no Pb or Bi, implying little if any diffusion loss from the inclusions.

As the X-ray data definitely show Pb (and sometimes Bi) in the 'spectacle' halo radiocentres, and as there is no evidence for any molecular ion contribution in the region from  $m/e =$

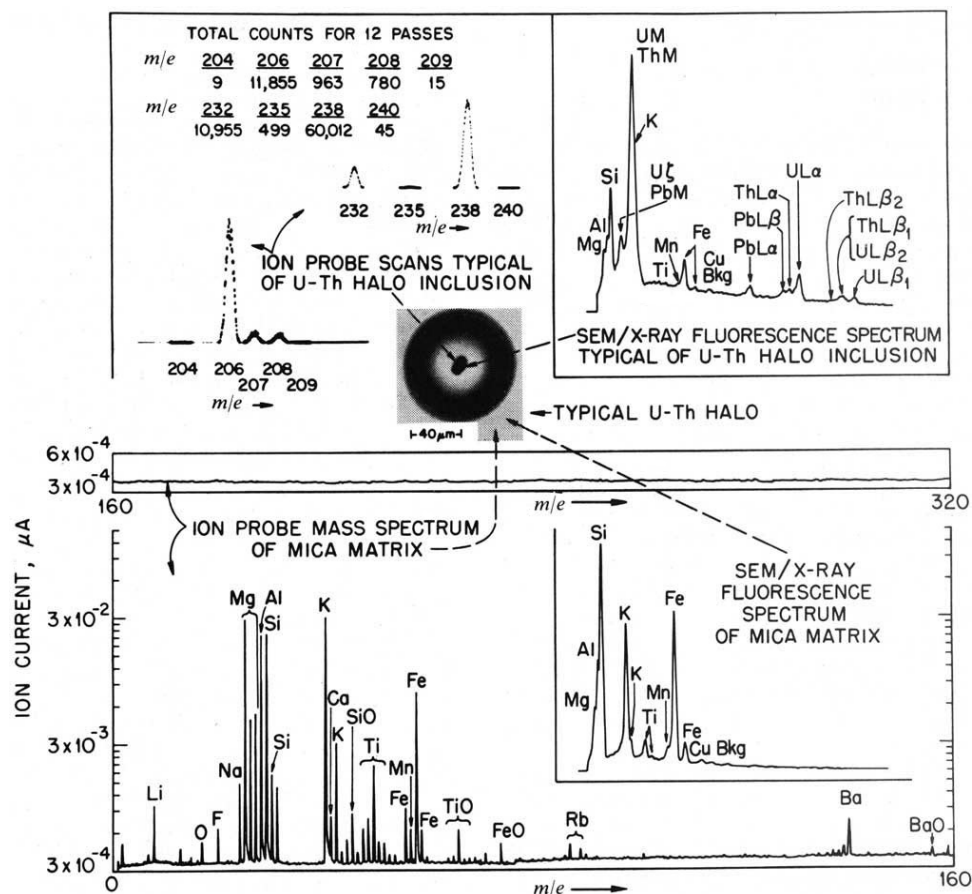


Fig. 2 Ion microprobe and XRF comparison between mica matrix and U-Th halo inclusion.

204–238, the 206, 207 and 208 peaks are interpreted as Pb isotopes and 209 as  $^{209}\text{Bi}$ ,  $^{204}\text{Pb}$ , a constituent of both common and primordial Pb, is missing (no 204 peak), implying that the 'spectacle' halo inclusions analysed contained no detectable Pb

of either of these types. Absence of the 232, 235 and 238 peaks is interpreted as showing the inclusions contain virtually no  $^{232}\text{Th}$ ,  $^{235}\text{U}$  or  $^{238}\text{U}$  and, therefore, no radiogenic  $^{206}\text{Pb}$ ,  $^{207}\text{Pb}$  or  $^{208}\text{Pb}$  derived from the *in situ* decay of these isotopes. The 207 and 208

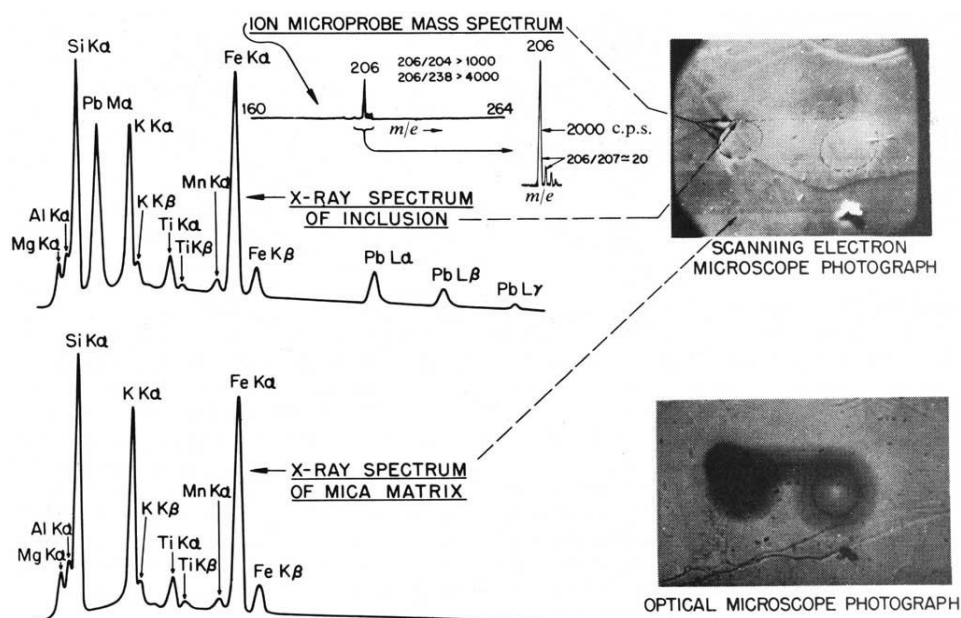


Fig. 3 Ion microprobe and SEMXRF spectra of mica matrix and  $^{210}\text{Po}$  halo inclusions.



peaks are therefore attributed to  $^{207}\text{Pb}$  and  $^{208}\text{Pb}$ , perhaps arising from the decay of minute amounts of  $^{211}\text{Bi}$  and  $^{212}\text{Bi}$  within the inclusions<sup>5,6</sup>. The  $^{209}\text{Bi}$  is considered to be primordial.

The outstanding feature of the mass analysis is the prominent 206 signal which, when attributed to the presence of  $^{206}\text{Pb}$  in the inclusions, fits perfectly with the prediction based on ring structure measurements, that is, that the  $^{206}\text{Pb}$  is radiogenically derived, not from U or Th, but directly from  $^{210}\text{Po}$   $\alpha$  decay. In this respect, the large difference in the 206/238 ( $^{206}\text{Pb}/^{238}\text{U}$ ) ratio between the 'spectacle' halo and the U-Th halo (Figs 2 and 3) is especially significant. Clearly the 'spectacle' halo resulted from  $^{210}\text{Po}$   $\alpha$  decay; an explanation for its geometry is still under study.

Because the Pb isotope in these inclusions is not explicable as any combination of common, primordial, or from *in situ* Pb derived radiogenically *in situ* from U or Th, we conclude that a different type of Pb, derived from Po  $\alpha$  decay, exists in nature. Supportive evidence comes from electron-probe and ion-probe analyses of a  $^{218}\text{Po}$  halo radiocentre found in a mica from the Iveland District, Norway, which yielded a  $^{206}\text{Pb}/^{207}\text{Pb}$  ratio of 23. This is consistent with that expected from  $^{218}\text{Po}$   $\alpha$  decay to  $^{206}\text{Pb}$ . Such a Pb ratio is impossibly high based on normal isotopic  $^{238}\text{U}/^{235}\text{U}$  decay, the theoretical maximum being 21.8.

Other investigations have shown varying mixtures of U-derived and Po-derived Pb may occur in the same radiocentre, for there exists an almost continuous halo spectrum stretching from 'pure' U to 'pure' Po haloes. Only a few (<0.01) Po haloes in biotite may survive the delicate sectioning process necessary for SEM X-ray analysis.

Just as important as the existence of a new type of lead is the question of whether Po haloes which occur in a granitic or pegmatitic environment (for example, in mica, fluorite or cordierite) can be explained by accepted models of Earth history<sup>1</sup>. (R. V. G. has found other  $^{210}\text{Po}$  haloes that differ essentially from those in granites—unpublished information.)

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## Gravitational driving and energy of Adriatic tides

IN small semiclosed seas, tides are excited through their open boundaries by the tides in the more extensive bodies with which they 'co-oscillate'<sup>1-5</sup>. Nevertheless the energy dissipated in such seas is not necessarily advected from and lost by, the larger system<sup>6</sup>.

In elongated gulfs opened to the ocean, such as the Bay of Fundy and the Gulf of California, exceptionally high tides may occur. In these two cases advection of energy from the ocean largely exceeds direct gravitational forcing<sup>6,7</sup>. The opposite situation may, however, also be encountered.

Although not spectacular, the tides in the Adriatic, which at the head reach 26 cm for the main semidiurnal constituent (M2) and 18 cm for the main diurnal constituent (K1), are also remarkable in view of the modest tidal amplitudes of the adjacent Ionian Sea (6.5 cm for M2 and 1.5 cm for K1)<sup>3</sup>. Semidiurnal dissipation in the Adriatic must be low<sup>8</sup> because of the effect of small amplitudes upon the faster than linear dependence of friction on velocity.

Although a square law frictional dependence must play an important role in reducing the tendency to dissipate at low amplitudes, inspection of the patterns of phases and amplitudes for M2 (cotidal and corange maps) suggests that the tidal flow is substantially in phase with the gravitational forces over most of the Adriatic. Thus, positive semidiurnal energy must be received directly from the gravitational forces balancing part or all of the frictional loss (Figs 1 and 2).

I believe that the Adriatic receives more than the energy it dissipates directly from gravitation at lunar semidiurnal frequency (M2). This conclusion is supported by calculations of energy budgets (see ref. 7) for the entire Adriatic, based on the ensemble of available observations, and a qualitative extension of considerations<sup>5</sup> about the position dependence of amphidromes (points of zero tide) upon dissipation.

Considering units of area transverse to the Adriatic, the

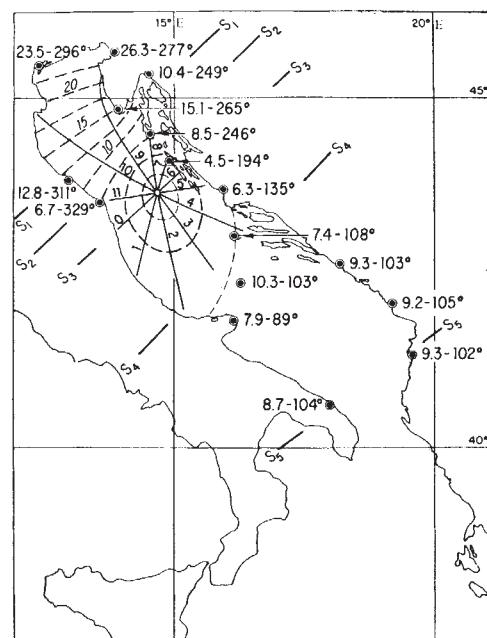


Fig. 1 Cotidal and corange maps for the semidiurnal constituent M2. Observed amplitudes and phases also shown. Cotidal lines (continuous) identified according to lag in hours behind equilibrium tide on meridian 15° E, corange lines (broken) labelled in cm.

**Table 1** Quantitative results from the Adriatic for semidiurnal constituent M2

| Section                                                 | Volume transport<br>( $\times 10^{11} \text{ cm}^3 \text{ s}^{-1}$ ) | Phase of transport*<br>(degrees) | Gravitational energy flux<br>( $\times 10^{14} \text{ erg s}^{-1}$ ) | Advection energy flux<br>( $\times 10^{14} \text{ erg s}^{-1}$ ) | Frictional dissipation<br>( $\times 10^{14} \text{ erg s}^{-1}$ ) |
|---------------------------------------------------------|----------------------------------------------------------------------|----------------------------------|----------------------------------------------------------------------|------------------------------------------------------------------|-------------------------------------------------------------------|
| S <sub>1</sub>                                          | 5.1                                                                  | 195                              |                                                                      |                                                                  |                                                                   |
| S <sub>2</sub>                                          | 6.4                                                                  | 194                              |                                                                      |                                                                  |                                                                   |
| S <sub>3</sub>                                          | 7.2                                                                  | 193                              |                                                                      |                                                                  |                                                                   |
| S <sub>4</sub>                                          | 5.4                                                                  | 197                              |                                                                      |                                                                  |                                                                   |
| S <sub>5</sub>                                          | 1.6                                                                  | 6                                | 1.2 to 2.45                                                          | -0.4 to -1.4                                                     | 0 - 2.05                                                          |
| Results for constituent K1, section S <sub>5</sub> only |                                                                      |                                  |                                                                      |                                                                  |                                                                   |
| S <sub>5</sub>                                          | 7.4                                                                  | 0-22                             | 0.15 to -0.45                                                        | 3.3 - 4.9                                                        | 2.85 - 4.75                                                       |

\* Refers to 0000 LT.

direct gravitational energy flux,  $E^g$ , is the sum of individual contributions according to

$$E^g = \sum_{n=1}^n \langle T_n F_n \Delta l_n \rangle \quad (1)$$

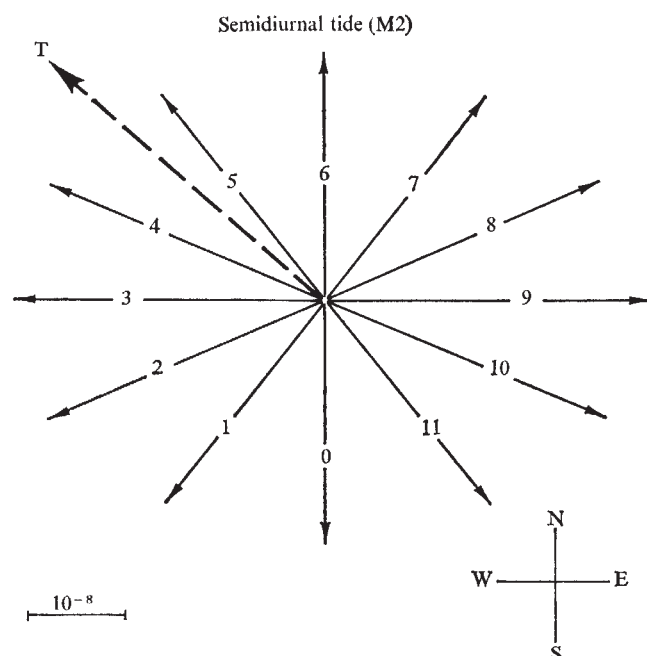
where  $T_n$  is volume transport across element  $n$ , covering distances  $\Delta l_n$ ; and  $F_n$  is the force which produces the gravitational tide.

The transport  $T_n$ , or volume crossing section  $n$  in a unit time equals the amount added to the prism  $P_n$ ; the latter represents the volume of water above mean sealevel to the head of section  $n$ , with

$$P_n = \sum_{n=1}^n H_n \Delta A_n, \quad T_n = dP_n/dt \quad (2)$$

where  $H_n$  is tidal elevation; and  $\Delta A_n$  is the horizontal area of section  $n$ . Thus,  $T_n$ , the time derivative of  $P_n$ , is  $90^\circ$  ahead of  $P_n$ .

The results (Table 1) indicate that over the major part of the Adriatic  $T$  remains close to its maximum value, occurring in the amphidromic area, and that it conserves nearly the same phase  $\Phi_T$ , a lag of roughly  $195^\circ$  or 6.5 h. (Phases are referred to  $15^\circ \text{ E}$  advance at an angular speed of  $\omega^{M2} = 2\pi/12$  radians, or  $30^\circ$ , per lunar hour).



**Fig. 2** Hourly variation of magnitude and geographical orientation of the Earth's surface slope with respect to equipotential surfaces for M2.  $T$ , direction of transport.

The tidal forces  $F$  (see equation 1) derive from the generating potential of the gravitational tide,  $W$ , according to

$$F = -\bar{\nabla} W \quad (3)$$

with, for the semidiurnal tide

$$W^{SD} = \rho g G^{SD} \cos^2 \theta \cos Z \quad (4)$$

where  $\bar{\nabla}$  is the horizontal gradient symbol;  $\rho$  is the specific mass of sea water;  $g$  is the acceleration of gravity;  $G^{SD}$  is the constant characteristic of each semidiurnal constituent;  $\theta$  is latitude and  $Z$  the hour angle, or longitude of lower transit of celestial body of interest—the Moon for constituent M2.

On a rigid Earth,  $G^{SD}$  for the semidiurnal constituent M2 is equal<sup>8</sup> to 24.3 cm. The elastic response of the Earth, however, depresses the tide potential by the factor  $(1 - h + k)$ , where  $h$  and  $k$  are the Love numbers characterising  $h$ , the solid Earth's response, and  $k$ , the resulting potential contribution<sup>9</sup>. Thus, the effective size of  $G^{SD}$  is reduced. The value  $(1 - h + k) = 0.69$  has been taken, leading to  $G^{SD} = 16.8 \text{ cm}$  for M2.

The hour-by-hour variation in magnitude and direction of the tidal slope over the Adriatic is shown in Fig. 2, together with the main geographical direction of transport—the Adriatic axis—and its phase.

Energy is supplied to the Adriatic tide when the tidal force component along the axis has the same direction as the transport. Work is done against the Moon in the opposite case. Over a tidal cycle both situations are encountered, but the gain exceeds the loss (Table 1). That can be visualised in Fig. 2.

The time-averaged influx of M2 gravitational energy is estimated at  $2.0 \times 10^{14} \text{ erg s}^{-1}$ . The main uncertainties are related to the coarseness of the integration scheme, neglect of motions transverse to the Adriatic, uncertainty on  $(1 - h + k)$ , and the possible influence of distortion of the Adriatic Basin through loading by the tide itself. The importance of transverse motion is commensurable with the minor-major axis ratio of the elliptical trajectories of the water particles if appropriate allowance for phase relationships is made. This all indicates an overestimation of  $E^g$  by a maximum of 20%. The cumulated uncertainty on the remaining factors could hardly exceed 20%. Thus, the M2 Adriatic tide receives, on an average,  $1.2\text{--}2.45 \times 10^{14} \text{ erg s}^{-1}$  directly from lunar gravitation.

Alternatively, advective driving by the Mediterranean tide is readily calculated by the flux method<sup>7</sup> according to the relationship:

$$E^a = \frac{1}{2} \rho g T H \cos \Phi_{T,H} \quad (5)$$

where  $T$  and  $H$  are volume transport and elevation, respectively, at the Adriatic-Mediterranean boundary; and  $\Phi_{T,H}$  is the phase difference between the two. Using the section between Brindisi and Durazzo, for which  $H$  is precisely known, as a boundary, equation (2) has led to  $T = 1.6 \times 10^{11} \text{ cm}^3 \text{ s}^{-1}$  and  $\Phi_{T,H} =$



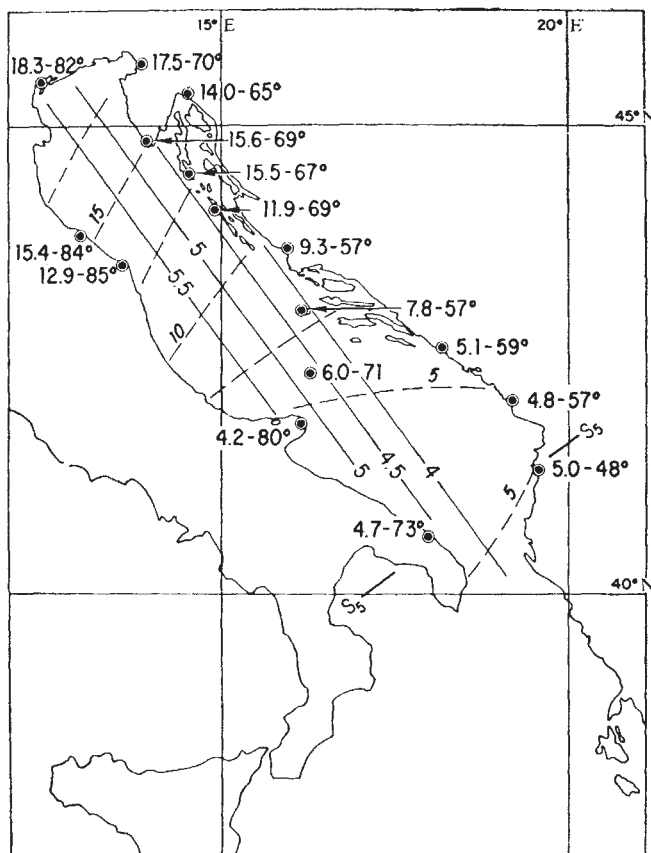


Fig. 3 Cotidal and corange map of the Adriatic for the diurnal constituent K1.

—98°, resulting in  $E^a = -10^{14}$  erg s<sup>-1</sup>. Thus, on the average, M2 energy is advected from the Adriatic to the Mediterranean. This fact not only confirms, but indirectly requires that gravitational energy be supplied to M2 tidal motions of the Adriatic.

It should be pointed out that  $T$  and  $\Phi_{T,H}$  and, therefore,  $E^a$ , are dependent on consideration of effects from the island cluttered north-eastern shore, and on selected cotidal and corange patterns. I have, therefore, taken careful account of island areas and optimised cotidal and corange maps. Those which I selected, based on Defant's data<sup>3</sup> (Fig. 1), do not differ significantly from those of Polli<sup>10</sup> which include more recent observations.

Estimates of tidal patterns show that displacement of the amphidrome toward the south-west reduces the energy flow to the Mediterranean. The sign changes for a 15 km displacement. Incompatibilities with the observations become noticeable, however, with a displacement of 7.5 km. Similar difficulties occur for north-eastward displacements of the order of 5 km. The energy transfer from the Mediterranean to the Adriatic thus falls between  $-0.4 \times 10^{14}$  and  $-1.4 \times 10^{14}$  erg s<sup>-1</sup>.

When the tide in a channel of constant cross-section can be represented by the sum of incident and reflected Kelvin waves with all the dissipation occurring in the reflection area, then amphidromic systems are formed with centres to the left of the axis in the northern hemisphere (the observer facing the head)<sup>5</sup>. This is valid in more general cases where dissipation also occurs along the channel. In such a case, however, the mathematical expression must differ.

In the Adriatic the evidence is for an amphidromic system very slightly displaced to the right rather than to the left (Fig. 1). This is even more evident if the north-eastern boundary is represented by a line displaced offshore from the main coastline in order to account for significant island area lost by the Adriatic Sea. Thus, here again, tidal energy loss from the Adriatic to the Mediterranean is implied.

Because conservative error bars have been accepted for  $E^a$  without providing for a possibility of advective energy flow toward the Adriatic, and because independent estimates of  $E^a$ , which are not sensitive to small changes in cotidal and corange maps, support the same conclusion, there must be a gain of direct semidiurnal gravitational energy.

Because of the uncertainty on both  $E^a$  and  $E^s$ , frictional dissipation within the Adriatic is not well established. It may fall anywhere between zero and  $2.05 \times 10^{14}$  erg s<sup>-1</sup>.

Tidal patterns for the main diurnal constituent, K1, are proposed on Fig. 3. Hour-by-hour variation of the gravitational slope can be derived from the diurnal potential:

$$W^D = \rho g G^D \sin 2\theta \cos Z \quad (6)$$

Treatment of the K1 tide along the same lines as the M2 tide shows that direct gravitational driving,  $E^a = -0.3 \times 10^{14}$  erg s<sup>-1</sup>,  $\pm 50\%$ , is negative but negligible compared with the energy influx through the Mediterranean boundary,  $E^s = 4 \times 10^{14}$  erg s<sup>-1</sup> ( $\pm 20\%$ ). Dissipation for K1 falls between 2.85 and  $4.75 \times 10^{14}$  erg s<sup>-1</sup>. Thus, it is appreciably higher than for M2, in spite of the greater amplitude and higher frequency of the latter.

Two factors peculiar to the Adriatic play an important role in its tidal behaviour: first, the closeness of its natural resonance to tidal frequencies, and second, the phase relationship between advective and gravitational driving. The interplay between these two factors allows semidiurnal tidal currents to flow downslope with respect to equipotential surfaces most of the time, and over most of the Adriatic, permitting the tide to gain appreciable energy in spite of the small size of the gravitational forces.

The closeness of the Adriatic to resonance is dramatically illustrated by the vulnerability of Venice to flooding<sup>10,11</sup>. The Adriatic's response to storms commonly results in oscillations—seiches—with near tidal periods which, combining with the tide, create catastrophically high waters—*aqua alta*. The near diurnal seich, with a period of about 22 h (ref. 10) is by far the most devastating. A near semidiurnal seiche is also observed, with a period around 11 h. Most of its energy escapes, however, into the Mediterranean in much the same manner that direct gravitational energy flux at semidiurnal frequency also leaks away. Thus, the highly damped near semidiurnal seiche has less disastrous effects.

Independent verification of the importance of direct gravitational driving of the Adriatic could be made by two approaches: mathematical modelling—analytical<sup>12</sup> or digital—and experimental investigation. Sufficiently precise positioning of the amphidrome would provide a useful, though mainly qualitative, answer. There are, however, difficulties resulting from the vanishing of the signal at the point of interest<sup>13</sup>. Alternatively, precise estimates of energy exchanges between the Mediterranean and Adriatic could be obtained by adequate current measurements through the Straits of Otranto.

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## Transition to CP conservation and zero Cabibbo angle in strong magnetic fields

Most current theories of particle symmetries assume that violations of these symmetries come about through a spontaneous breaking mechanism which produces non zero expectation values for certain scalar (elementary or composite) fields. As is well known (refs 1–7 and Lebedev Institute Reprint No. 101) these expectation values may make a phase transition to a zero value for certain critical temperatures and possibly also for certain critical external magnetic field strengths  $H_c$ ,  $H_{c_2}$ , ... Here we point out that it is conceivable that the charge asymmetry (associated with CP violation) in  $K_L \rightarrow \pi^\pm + l^\pm + \bar{\nu}(v)$  decays may disappear for fields of  $\sim 8 \times 10^{10}$  gauss if CP violation is milli-weak in character, and that the Cabibbo angle may be reduced to zero—leading to suppression of certain hyperon decays—in fields of the order of  $10^{16}$  gauss. These estimates are so strongly model-dependent that it may be worthwhile in any case, to make a systematic phenomenological search for effects on particle asymmetries of strong magnetic fields of  $10^6$  gauss upwards.

The notion of critical fields (above which spontaneously broken symmetries are restored) is well known from the Ginzburg–Landau theory of superconductivity which is also the prototype of spontaneous symmetry-breaking mechanisms used in particle physics. An order of magnitude estimate of the critical field  $H_c$  in this theory is provided by considering the free energy:

$$F = F_n + \alpha(T)|\phi|^2 + \frac{\lambda(T)}{2}|\phi|^4 + \dots \quad (1)$$

where  $n$  denotes normal.

For temperature  $T < T_c$ ,  $F$  has a minimum when

$$|\langle \phi \rangle|^2 = -\alpha/\lambda \quad (2)$$

The 'thermodynamic' critical field close to  $T_c$  is then given by<sup>8</sup>:

$$\begin{aligned} \frac{H_c^2(T)}{8\pi} &= F_n - F_s \\ &= \frac{1}{2} \frac{\alpha^2}{\lambda} \\ &= \frac{1}{2} \lambda |\langle \phi \rangle|^4 \end{aligned} \quad (3)$$

where  $s$  denotes superconducting.

Now in superconductivity theory the (Cooper-pair) field  $\phi$  is itself charged and the magnetic field interacts directly with it. For  $K^0$  fields (which we wish to investigate here) this is not the case. In an  $SU(3)$ -symmetric theory, however, the magnetic field does interact in higher order loop graphs. To see the qualitative emergence of a formula like equation (3), we later consider (for  $T = 0$ ) an  $O(3)$ -symmetric gauge model, where the electromagnetic field is part of a gauge-triplet. Even though we are not dealing with a thermodynamic system, a critical field  $H_c$  seems to exist in a one-loop approximation above which the

expectation value of the neutral field  $\langle \phi_3 \rangle$  vanishes—the relationship between  $H_c$  and  $\langle \phi_3 \rangle$  having the general form of equation (3). Here we shall use this formula for order of magnitude estimates. Clearly  $H_c$  will be small for those situations where  $\langle \phi \rangle$  and  $\lambda$  are small.

Now for strong interaction symmetries like  $SU(2)$  or  $SU(3)$ , values of  $\langle \phi \rangle$  are typically  $\sim$  BeV (or somewhat less), with  $\lambda \simeq 1$ . But for weak interaction symmetries—and these provide the more spectacular physical situations—with universal gauge coupling of order unity ( $g^2 \approx 4\pi/137$ ,  $g \approx 1/3$ ),  $\langle \phi \rangle$  values are typically tens or hundreds of BeV in magnitude, except for 'off-diagonal' situations. By way of illustration we consider the  $\langle \phi \rangle$  associated with real rotations of the  $n$ - $\Lambda$  system (Cabibbo angle) and  $\langle \phi \rangle$  associated with complex rotations which signal CP violation in a milli-weak manner.

To estimate the magnitude of  $H_c$  for these cases, assuming that it exists we consider the ideas of Lee<sup>9</sup>, Pais and Primack<sup>10</sup> or Mohapatra and Pati (ref. 11 and University of Maryland Technical Report No. 74-O85). In their milli-weak theory, the latter authors write the mass matrix for the  $n$ ,  $\Lambda$  quark system in the form:

$$f(\bar{n}, \bar{\Lambda})_L U_L \begin{bmatrix} m_n & 0 \\ 0 & m_\Lambda \end{bmatrix} V_R^{-1} \begin{bmatrix} n \\ \Lambda \end{bmatrix}_R \quad (4)$$

where  $U_L$  and  $V_R$  are matrices of the type:

$$\begin{vmatrix} \cos \theta_{L,R} & -\sin \theta_{L,R} \exp(i\delta_{L,R}) \\ \sin \theta_{L,R} \exp(i\delta_{L,R}) & \cos \theta_{L,R} \end{vmatrix} \quad (5)$$

with  $\theta$  and  $\delta$  real. The real part of the off-diagonal elements of the expectation-value matrix

$$U_L \begin{bmatrix} m_n & 0 \\ 0 & m_\Lambda \end{bmatrix} V_R^{-1}$$

(assuming for simplicity  $\theta_L = \theta_R$ ) is given by:

$$\frac{1}{2}(m_\Lambda - m_n) \sin 2\theta_c \cos(\varepsilon/2) \quad (6)$$

where  $\delta_L = \varepsilon/2$ ,  $\delta_R = -\varepsilon/2$ . Now from CP violation,  $\varepsilon$  in this model can be as small as  $2 \times 10^{-3}$ . With  $m_\Lambda - m_n \simeq 175$  MeV,  $\theta_c \sim 15^\circ$  and assuming  $f$  in equation (4) to be typically  $\simeq 1$ , we obtain:

$$\text{Re} \langle \phi \rangle |_{\text{off-diagonal}} \simeq 45 \text{ MeV} \quad (7)$$

Using equation (3) and making the *ad hoc* assumption that the numerical constant ( $\lambda$ ) appearing there is of order unity, we obtain

$$H_c \simeq 3 \times 10^{16} \text{ gauss} \quad (8)$$

(using the conversion  $\langle \phi \rangle$  (MeV) =  $0.3H$  (kgauss) cm).

If fields of this magnitude are applied, the real part of the 'off-diagonal' expectation value may vanish and, with it, the Cabibbo angle, suppressing certain hyperon and strange-particle decays.

Also, the imaginary part of the off-diagonal matrix element of the mass matrix is

$$(f/2)(m_\Lambda + m_n) \sin 2\theta_c \sin$$

This expectation value may make a transition to a zero value for fields  $H_c \simeq 10^{14}$  gauss—and with it the (milli-weak) CP asymmetry—for a heavy quark model ( $m_\Lambda \sim 5$  BeV); for a light quark model ( $m_\Lambda \sim 300$  MeV), on the other hand,  $H_c$  may reduce to  $8 \times 10^{10}$  gauss.

(A preliminary and perhaps unrealistic model for spontaneous superweak CP breaking seems to give a value of around  $3 \times 10^6$  gauss for the critical field strength. This is likely to be a very gross—though morale-building—underestimate, from the experimental point of view.)



Since in these estimates we have assumed, for simplicity, that all model-dependent constants like  $f$  and  $\lambda$  are  $\sim$  unity and, further, since equation (3) itself is a very crude estimate, it is clear that  $H_c$  may change by orders of magnitude, and also not one but a hierarchy of critical fields may be discovered when a detailed investigation is carried through. This situation is not unfamiliar in superconductivity theory, where, depending on the ratio of the parameters  $\lambda$  and  $g$  (the gauge coupling), superconductors are divided into Type I and Type II, with Type II displaying a variety of critical fields<sup>12</sup>  $H_c$ ,  $H_{c1}$ ,  $H_{c2}$ ,  $H_{c3}$  and a corresponding variety of physical (vorticity) characteristics (For  $V_3\text{Ga}$ , for example, there are three widely differing critical fields,  $H_{c1}$  ( $T=0$ )  $\simeq 200$  gauss,  $H_c$  ( $T=0$ )  $\sim 6,000$  gauss, and  $H_{c2}$  ( $T=0$ ) which is as large as 300,000 gauss.)

Detailed models, in which we shall investigate the analogues of fields  $H_{c1}$ ,  $H_{c2}$ , ... for particle physics, will be considered elsewhere.

Fields of strength  $\sim 10^5$  gauss are possibly technically feasible for use in conjunction with K-beam experiments. Clearly, in order to test the ideas expressed here, stronger fields will be needed. There is no doubt, however, that if the basic ideas of spontaneous symmetry breaking are correct and if we believe in the validity of extrapolating from the one-loop calculation presented later, then there would exist critical fields for which the broken symmetries are likely to be restored. The future task of the theory is to explore such situations where the field strength required is not excessively intense and within reach of the foreseeable technology. Since it is commonly assumed that the mean magnetic fields in pulsars are  $\simeq 10^{12} \simeq 10^{14}$  gauss, it would seem—for what it is worth—that CP-violating phenomena do not take place in pulsars, though the Cabibbo angle is still non-zero.

To illustrate the effect of a uniform magnetic field on symmetry breaking, we give a simple model calculation. A Lagrangian which exhibits local  $O(3)$  symmetry is given by:

$$\mathcal{L} = \frac{1}{4} \mathbf{F}_{\mu\nu}^2 + \frac{1}{2} (\nabla_\mu \boldsymbol{\varphi})^2 + \frac{\mu^2}{2} \boldsymbol{\varphi}^2 - \frac{\lambda}{4} (\boldsymbol{\varphi}^2)^2$$

where the scalar and vector fields,  $\boldsymbol{\varphi}$  and  $\mathbf{A}_\mu$ , are triplets with respect to the  $O(3)$  symmetry and the covariant derivatives which appear are

$$\begin{aligned} \nabla_\mu \boldsymbol{\varphi} &= \partial_\mu \boldsymbol{\varphi} + e \mathbf{A}_\mu \times \boldsymbol{\varphi} \\ \mathbf{F}_{\mu\nu} &= \partial_\mu \mathbf{A}_\nu - \partial_\nu \mathbf{A}_\mu + 2e \mathbf{A}_\mu \times \mathbf{A}_\nu \end{aligned}$$

The parameters  $\mu^2$  and  $\lambda$  are both positive: this implies a symmetry breakdown,  $O(3) \rightarrow O(2)$ , in the tree approximation. The vacuum expectation value of  $\boldsymbol{\varphi}$  is non-vanishing, and indeed, may be used to define a direction in iso-space

$$\langle \boldsymbol{\varphi} \rangle = \sqrt{(\mu^2/\lambda)} \delta^{13}.$$

The corresponding component of the gauge potential,  $A_\mu^3$ , does not acquire a mass and so may be identified with the electromagnetic potential. In the tree approximation the presence of an external background electromagnetic field,  $\langle A_\mu^3 \rangle \neq 0$ , has no influence on the expected value of the neutral component  $\langle \varphi^3 \rangle$  of the matter triplet.

When quantum corrections are taken into account this is no longer true. Already, the one-loop corrections to the effective action, given formally by:

$$\Gamma_{(1)}(\boldsymbol{\varphi}, A) = (\hbar/2i) \ln \text{Det} G_{(0)}(\boldsymbol{\varphi}, A)$$

(where  $G_{(0)}$  denotes the set of classical propagation function for small disturbances on a given background) imply a coupling between  $\varphi^3$  and  $A_\mu^3$  even when the charged components  $\varphi^{1,2}$  and  $A_\mu^{1,2}$  are set equal to zero in the modified ground state.

In the uniform magnetic background, where all fields vanish

except for

$$\varphi^3(x) = \psi, \quad A_1^3(x) = \frac{1}{2} H x_2, \quad A_2^3(x) = -\frac{1}{2} H x_1$$

with  $\psi$  and  $H$  constant, the one-loop contribution to the effective potential,  $V_{(1)}(\psi, H)$ , is determined approximately by the differential formula

$$\begin{aligned} 2 \frac{\partial V_{(1)}}{\partial \psi^2} &= \frac{\lambda \hbar}{8\pi} \int_0^\infty \frac{ds}{s} \left[ \left\{ \frac{eH}{\sinh(seH)} \exp[-s(\lambda\psi^2 - \mu^2)] - \frac{\exp[-s(\lambda M^2 - \mu^2)]}{s} (1 - s\lambda(\psi^2 - M^2)) \right\} + \right. \\ &\quad \left. + \frac{3}{2\pi s} \left\{ \exp[-s(3\lambda\psi^2 - \mu^2)] - \exp[-s(3\lambda M^2 - \mu^2)] \times \right. \right. \\ &\quad \left. \left. \times (1 - 3s\lambda(\psi^2 - M^2)) \right\} \right] \end{aligned}$$

(This expression is renormalised at  $H=0$ ,  $\psi=M$ , that is,  $\partial V_{(1)}/\partial \psi^2$  and  $\partial^2 V_{(1)}/\partial (\psi^2)^2$  both vanish at this point.) The formula is approximate because we have not included the contributions due to intermediate vectors. The term with the  $H$ -dependent factor here is the contribution to  $V_{(1)}$  of the intermediate charge pair  $\varphi^{1,2}$ , whereas the  $H$ -independent factor is due to the neutral  $\varphi^3$ .

To determine the critical field, we should set  $\psi=0$  and require that  $2\partial V_{(1)}/\partial \psi^2|_{\psi=0}$  should compensate the tree contribution  $2\partial V_{(0)}/\partial \psi^2|_{\psi=0} = -\mu^2$ . Unfortunately, the neutral particle contribution to  $\partial V_{(1)}/\partial \psi^2$  is complex if  $\psi^2 < \mu^2/3\lambda$ . This means that our approximation is inadequate. The source of this complexity is easily traced.

In restricting ourselves to one-loop effects we are not allowing the magnetic field to act on the neutral intermediate particles. Hence when  $\psi=0$  these appear to carry imaginary mass,  $i\mu$ . The very object of the computation  $\partial V/\partial \psi^2 = 0$ , is however, the setting to zero of the neutral particle mass. Clearly, we should be setting up a self-consistent (Dyson) equation: which means bringing in the contributions of higher loops. (For a similar pathology in a calculation of critical temperature see the paper of Dolan and Jackiw<sup>5</sup>.) Short of this, we can obtain an order of magnitude idea of the critical field by setting both  $\mu$  and  $\psi$  to zero specifically in the above expression for  $\partial V_{(1)}/\partial \psi^2$ . That is, we solve the restricted problem

$$\begin{aligned} 0 = -\mu^2 + \frac{\lambda \hbar}{8\pi} \int_0^\infty \frac{ds}{s} \left[ \left\{ \frac{eH_c}{\sinh(seH_c)} - \frac{\exp(-s\lambda M^2)}{s} (1 + s\lambda M^2) \right\} \right. \\ \left. + \frac{3}{2\pi s} \left\{ 1 - \exp(-3s\lambda M^2) (1 + 3s\lambda M^2) \right\} \right] \end{aligned}$$

or

$$\begin{aligned} \frac{\mu^2}{M^2} &= \frac{\lambda^2 \hbar}{8\pi} \int_0^\infty \frac{du}{u} \left[ \frac{eH_c/\lambda M^2}{\sinh(ueH_c/\lambda M^2)} - \exp(-u) \left( \frac{1}{u} + 1 \right) \right. \\ &\quad \left. + \frac{3}{2\pi} \left\{ \frac{1}{u} - \exp(-3u) \left( \frac{1}{u} + 3 \right) \right\} \right] \\ &= \frac{\lambda^2 \hbar}{8\pi} \left[ a + b \frac{eH_c}{\lambda M^2} \right], \end{aligned}$$

where

$$\begin{aligned} a &= 1 + \frac{9}{2\pi} \left\{ \right. \text{and } b = \int_0^\infty \frac{dv}{v} \frac{d}{dv} \left( \frac{v}{\sinh v} \right) \left. \right\} \\ &\simeq 2.5 \qquad \qquad \qquad \simeq -1 \end{aligned}$$

On choosing the reference mass equal to  $\mu$  we obtain

$$H_c = \frac{1}{b} \left( \frac{8\pi}{\lambda^2 \hbar} - a \right) \frac{\lambda \mu^2}{e} \\ = \frac{1}{b} \left( \frac{8\pi}{\lambda^2 \hbar} - a \right) \frac{\lambda^2 \langle \phi \rangle^2}{e}.$$

Since  $b$  is negative, we must have  $\lambda^2 \hbar > 8\pi/a$ .

This estimate of  $H_c$  should not be taken as anything more than a rough indication. The form of this expression for  $H_c$  is basically similar to equation (3), apart from the numerical factor multiplying  $\langle \phi \rangle^2$ . It is factors of this type which in a realistic calculation may drastically alter the order of magnitude estimates given earlier. Also, for a situation involving a number of non-zero  $\langle \phi \rangle$ s, one may conjecture that  $H_c$  would be an expression involving a sum of terms like  $\langle \sum f_i \phi_i \rangle^2$  with sequences of opposing signs among the coefficients  $f_i$ . This, in turn, may lead to the desired diminution of the absolute magnitudes of the critical field strengths.

It is perhaps worth remarking that the calculation presented above for the critical field does not constitute a general proof that such fields always exist. This problem clearly needs further study.

We thank Professor D. J. Bradley for pointing out that fields of  $10^9$  gauss in a small volume  $\sim 10^{-9} \text{ cm}^3$  lasting more than  $10^{-9}$  s may conceivably be produced by laser compression techniques (though improvements in laser technology could permit the extension of both volume and duration) and to Dr Delbourgo for the remark that laser compression would also be accompanied by a high temperature which may help to produce the critical magnetic field at the same time. There is the further possibility that the electromagnetic energy pumping by the laser beam may by itself achieve the same purposes as are described in this communication. Such laser beams would have to have enormous powers of  $\sim 10^{18} \text{ W cm}^{-2}$ .

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The precise nature of the heavy metal fallout, however, has been neglected although the chemical and physical state of a metal determine to a large extent its mobility in the soil and hence its availability to plants and animals.

We have, therefore, trapped air particulates and examined them by scanning electron microscopy, together with an energy dispersive Kevex X-ray analyser attached to a similar microscope. Thus it was possible to select particles down to approximately  $1 \mu\text{m}$  diameter.

Air particulates were trapped on strips of adhesive aluminium tape mounted on aluminium backing plates, freely suspended at about shoulder height in the vicinity of the industrial complex, in comparable positions to those previously used<sup>1</sup>. After several weeks, 1-cm circles were cut from the tapes and mounted on electron microscope stubs. These were then vacuum coated with a thin layer of aluminium to prevent 'charging' under the microscope beam.

Debris trapped on the tapes ranged from small flies to spherical particles of less than  $1 \mu\text{m}$  diameter. In particular, a large number of spherical particles ( $< 1 \mu\text{m}$ – $10 \mu\text{m}$ ) were observed under the light microscope. Because of their perfect symmetry, they were thought to be metallic droplets condensed from the vapour in furnace chimneys and flues (E. R. Buckle, and K. C. Poinon, unpublished). X-ray spectra of these particles, however, showed the majority of them to be constituted predominantly of silicon with associated sodium, potassium, calcium, iron, vanadium and titanium in varying amounts. A smaller number of particles had relatively large levels of iron. Similar spherical balls were observed on leaf samples from vineyards belonging to Long Ashton Research Station. Two apparently identical balls  $3 \mu\text{m}$  diameter and about  $15 \mu\text{m}$  apart on a leaf were analysed. The first gave the normal silicon type spectrum while the second showed only the  $K\alpha$  and  $K\beta$  iron peak with no other metals being identified (Fig. 1a and b).

The spectra of less regular particles were examined. Most were of the general silicon type (Fig. 1a) but a few rather more crystalline samples gave strong sulphur and calcium peaks (Fig. 1c). Others gave high sodium, chlorine or calcium peaks while one particle seemed to consist solely of sulphur. Of the three elements of most direct interest, that is, cadmium, lead and zinc, particles containing the latter were the most abundant ( $0.5$ – $10 \mu\text{m}$  in diameter). These particles also contained silicon, calcium, potassium, sodium and sulphur (Fig. 1d). Other particles, however, were found to consist solely of zinc and sulphur (Fig. 1e). The Kevex energy dispersive detector does not detect oxygen which may or may not be present in these particles.

Lead occurred much less frequently than zinc, but was generally in particles in the  $50$ – $100 \mu\text{m}$  range. It was found associated with small amounts of zinc and cadmium (Fig. 1f). Cadmium was found only in isolated cases and always associated with lead.

Analyses of the tapes by acid digestion and atomic absorption spectrophotometry gave zinc/lead/cadmium ratios similar to those found in vegetation<sup>1</sup> or moss bags<sup>2</sup>. The larger size of the lead-containing particles accounts for the much greater number of zinc particles, since the absolute amounts of the two metals is in the approximate ratio of 2 of zinc to 1 of lead.

Only a small number of the total number of particles trapped have been examined so far; hence conclusions must be tentative. Also the particles examined may not be truly representative of all fallout particles from the complex.

But these results seem to suggest the presence of zinc in two forms: (1) in the presence of silicon, presumably as a form of silicate, and (2) in the presence of sulphur, either as sulphide or sulphate. As the Kevex detector will not respond to elements below atomic number 11 we cannot at this stage distinguish between a sulphide and sulphate.

As lead was found in the presence of varying amounts of zinc and cadmium but not with silicon or sulphur, it may be present as some form of oxide. Cadmium has been found only

## Heavy metal particle characterisation

THE aerial fallout of heavy metals downwind of the Avonmouth industrial complex has been well documented. Analyses of vegetation<sup>1</sup> and moss bags<sup>2</sup> show decreasing levels of lead, cadmium and zinc with increasing distance from the complex.



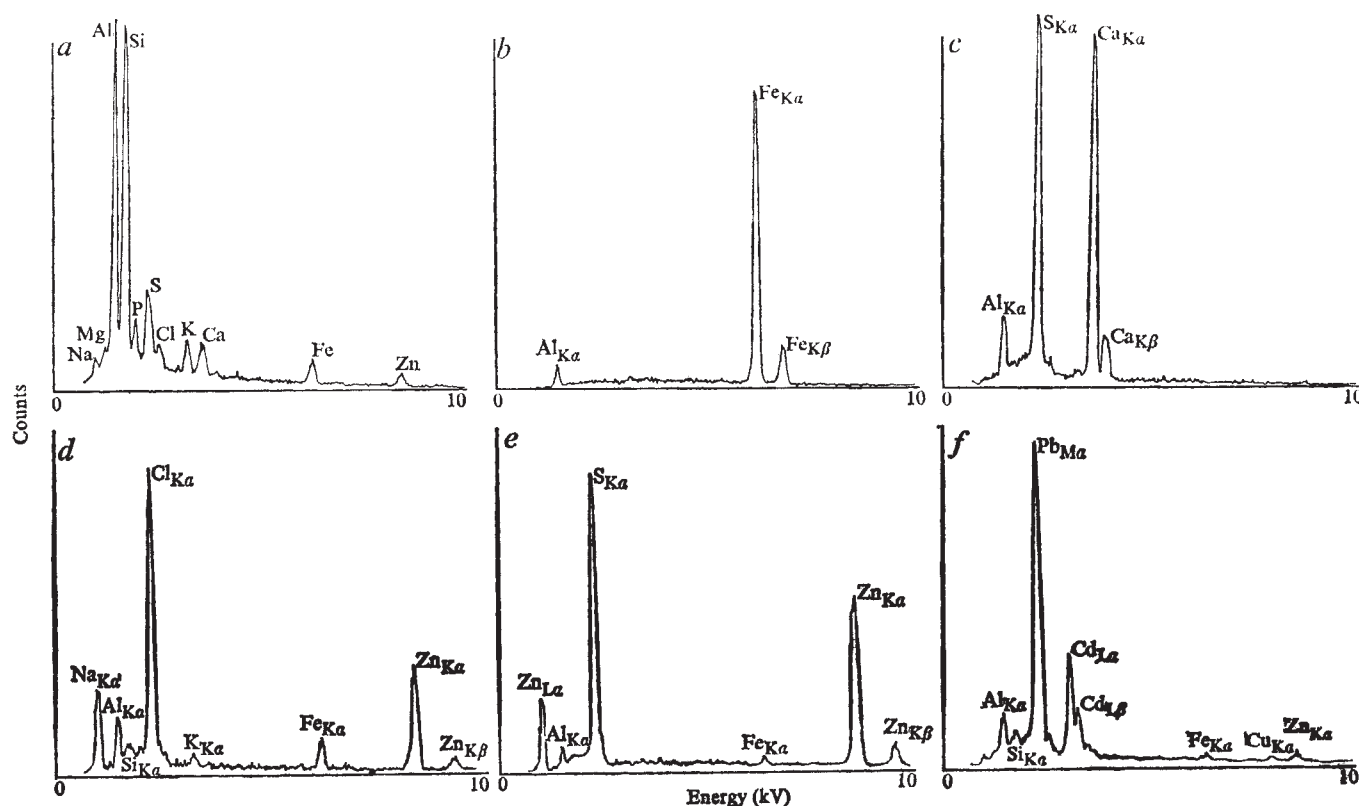


Fig. 1 X-ray emission spectra. *a*, Typical silicon-type particle; *b*, iron-containing particle; *c*, crystalline particle; *d*, zinc and silicon-containing particle; *e*, zinc and sulphur-containing particle; *f*, lead and cadmium-containing particle.

rarely and always in the presence of lead and at much lower levels.

Unfortunately, since our method is only qualitative, the relative ratios of the elements in different particles cannot be determined. Using ESCA techniques it may be possible to determine the oxidation state of the elements such as zinc, lead and sulphur within the particles. This would then offer a more meaningful indication of the exact chemical composition of the fallout. This work is now in progress.

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## Residence time of sphere or air bubble in sheared non-Newtonian fluids

DURING experiments to measure the residence time of air bubbles in a stirred beaker, we observed that for polyacrylamide solutions the bubbles rose more slowly as rotational speed was increased (Fig. 1). This result contradicted our expectation that 'shear thinning' of a non-Newtonian fluid would result in a more rapid rise of the bubbles. The multiple-rod impeller used in these initial experiments was similar in design to that used by Steel and Maxon<sup>1</sup> for aeration of a novobiocin-producing actinomycete. Single air bubbles of known volume were generated by a Chromatronix 4-way valve; bubble size could be changed by changing the size or length of the splitting loop on the valve, and the volume of the bubble was checked by measuring its length in a capillary tube of known cross-sectional area. The rise of air bubbles was traced by high speed film.

This unexpected phenomenon was further investigated in a modified Couette viscosimeter device. The bob, 3.85 cm in diameter, was driven by a variable-speed motor while the cup, 11.20 cm in diameter, was stationary. The height of solution in

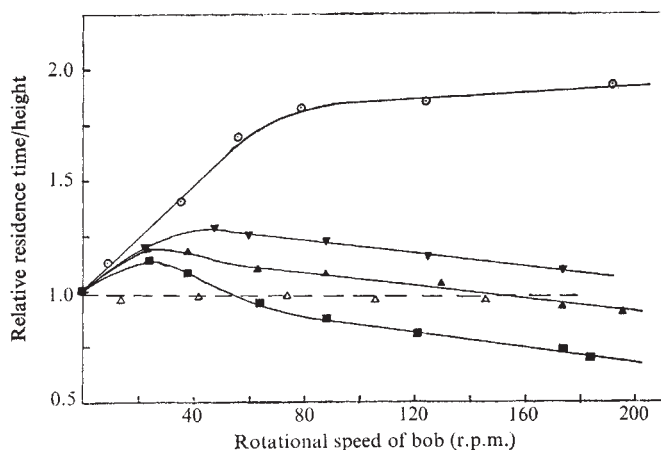
Table 1 Residence time as a function of radial distance for falling spheres

| Polymer solution                      | 0.6% Polyhall |       | 1.0% Polyox |       |
|---------------------------------------|---------------|-------|-------------|-------|
| Radial distance from bob surface (cm) | 0.52          | 1.85  | 1.17        | 1.85  |
| $\theta$ (s cm <sup>-1</sup> )        | 0.878         | 0.752 | 0.607       | 0.579 |
| Bob rotational speed (r.p.m.)         | 8             |       | 84          |       |
| Aluminium sphere diameter (mm)        | 4.76          |       | 3.17        |       |

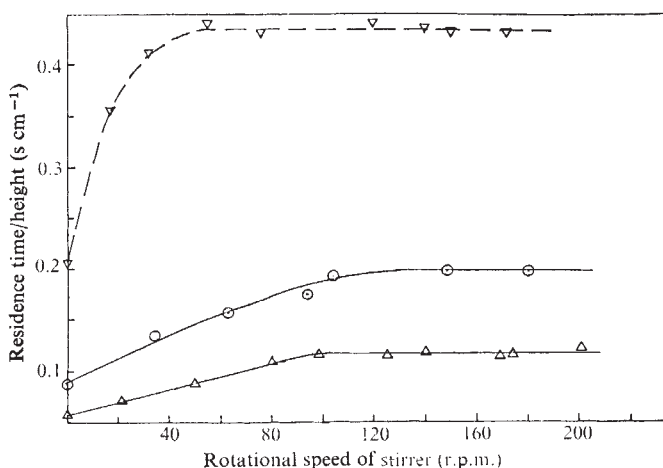
the annular space was 21 cm. Because there was distortion of 4% and 12% on the bubble's minor and major axes as the multiple-rod impeller swept past the bubbles, rigid aluminium spheres were also used to eliminate the possibility that deformation of the bubble was a cause of the observed effect. Residence time per unit height was found by observing the time of rise or fall for six to ten repeated runs through a fixed distance of 10 cm. The relative residence time,  $\theta_r$ , is the ratio of the residence time in a sheared solution to that in a stagnant solution. Two pseudo-plastic polymer solutions were investigated: 0.6% aqueous polyacrylamide (Polyhall 295) and 1.0% aqueous polyethylene oxide (Polyox WSR-301).

When air bubbles were released at the same radial position, the relative residence time increased with increasing shear rate and eventually reached an asymptotic value for both Polyhall 295 and Polyox solutions (Figs 2 and 3). Control tests with glycerol, a Newtonian liquid, showed that  $\theta_r$  was independent of the shear rate, as it should be. These observations confirmed what had been found for the multiple-rod stirrer. On the other hand, for falling rigid spheres in the Couette device a maximum in  $\theta_r$  was noted. For small spheres at sufficiently high speeds of the bob a shear-thinning effect did occur, but at lower speeds and for larger spheres there was again an abnormally high residence time.

It is difficult to account for this behaviour. Secondary flows can occur in the annulus of a Couette device (Taylor instability), especially when the inner rather than the outer cylinder is



**Fig. 2** Relative residence time per unit height in 0.6% Polyhall solution.  $\odot$ , Rising air bubble in 0.6% Polyhall;  $\triangle$ , in glycerol ( $0.452 \text{ N s}^{-1} \text{ m}^{-2}$  (452 cpoise at  $22.5^\circ \text{C}$ ). Both at  $22.5 \pm 0.2^\circ \text{C}$ . Air bubble diameter was  $5.13 \pm 0.02 \text{ mm}$ . Aluminium spheres with diameters:  $\blacksquare$ , 1.59 mm;  $\blacktriangle$ , 3.17 mm;  $\nabla$ , 4.76 mm, were released at the same radial distance from centre ( $r = 3.3 \text{ cm}$ ) at  $25.2 \pm 0.2^\circ \text{C}$ . Dashed line, Newtonian behaviour.



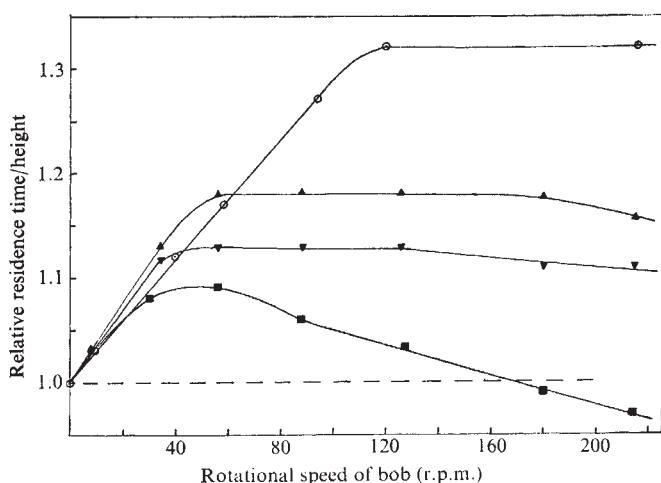
**Fig. 1** Residence time per unit height for air bubbles in Polyhall 295 solutions stirred by multiple-rod stirrers. The concentration of Polyhall solutions were:  $\triangle$ , 0.2%;  $\odot$ , 0.3%;  $\nabla$ , 0.5%. Air bubble diameter was  $5.12 \pm 0.02 \text{ mm}$ , and the temperature of the solution was  $22.5 \pm 0.5^\circ \text{C}$ . Bubbles were released at the mid point between the shaft and the wall.

rotated<sup>2</sup>. No such secondary flow was expected or was observed at the shearing rates used here, however, an end effect at the bottom was observed in the Polyhall 295 solution, but the torus-shaped circulation never extended above 6 cm from the bottom. The end effect did not influence the results because the timed distance for the fall of the spheres was always above this level. The anomalous shear 'thickening' effect may result from the asymmetrical flow field around a rising bubble or falling sphere; the normal stress distribution may be such as to create a net opposing elastic force. As the particle diameter increases, deviation from the ideal flow field becomes more prominent and the normal stress effect is more noticeable.

A further observation which confirms the increase in apparent transverse viscosity with increasing rate of shear is summarised

in Table 1. For a given size of sphere and angular velocity of the bob, the residence time increased as the radial distance between the point of release of the sphere and the bob decreased. The highest shearing rate is next to the rotating bob and, therefore, the residence time should have decreased in the vicinity of the bob if the polymer had a shear thinning property when the sphere was travelling transverse to the shear field. In qualitative terms, the chains of polymer molecules can be visualised as more uniformly aligned near the rotating bob than distant from it. Thus, a falling sphere penetrates the random molecular array at some distance from the bob faster than it penetrates the oriented or 'structured' molecules close to the rotating bob.

Highgate and Whorlow<sup>3</sup> found only slightly anomalous behaviour in a sphere falling through a sheared solution of 10% polyisobutylene in tetralin, another non-Newtonian system. They observed a decrease in 'transverse viscosity' as the shear



**Fig. 3** Relative residence time per unit height in 1.0% Polyox WSR-301 solution.  $\odot$ , Rising air bubble with diameter  $5.13 \pm 0.02 \text{ mm}$  at  $24.7 \pm 0.2^\circ \text{C}$ . Aluminium spheres with diameters:  $\blacksquare$ , 1.59 mm;  $\nabla$ , 2.50 mm;  $\blacktriangle$ , 3.17 mm, were released at the same radial distance from the centre ( $r = 3.3 \text{ cm}$ ) at  $24.5 \pm 0.2^\circ \text{C}$ . Dashed line, Newtonian behaviour.



rate was increased, but the thinning effect was not as large as would be predicted from conventional measurements assuming that viscosity is an isotropic property. Perhaps still other systems and geometries need to be tested to ensure that the effect is not an artefact and to explore its dependence on the molecular properties that give rise to non-Newtonian behaviour.

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## Polymeric film precursors in the homogeneous crystallisation of some metal oxides

We have already reported<sup>1,2</sup> the occurrence of two-dimensional polymeric structures prior to precipitation in the system  $\text{ZrOCl}_2$  (aqueous solution)  $\rightarrow$   $\text{ZrO}_2$  (monoclinic). Here we show that similar thin polymeric films are precursors in the precipitation of  $\alpha\text{FeOOH}$ ,  $\beta\text{FeOOH}$ , and  $\text{V}_2\text{O}_5 \cdot \text{H}_2\text{O}$ , and that the morphology

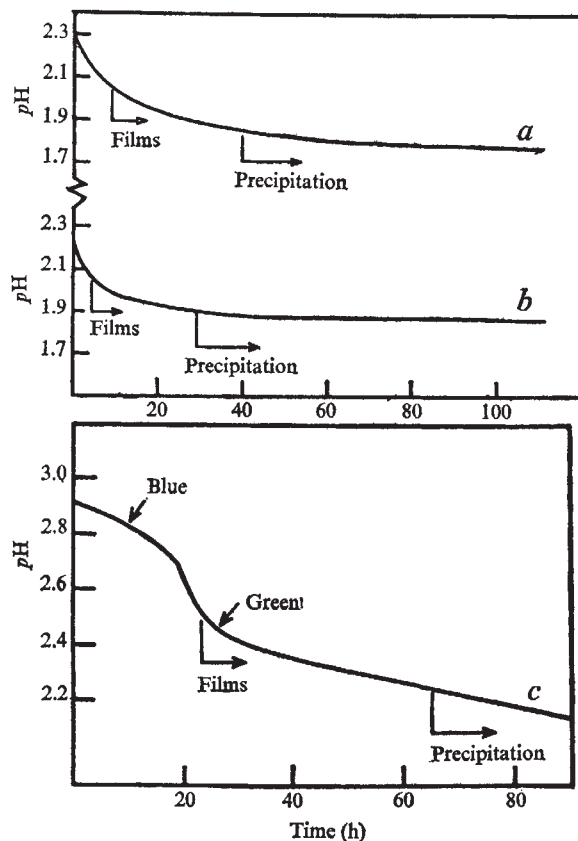


Fig. 1 Dependence of pH on time for the hydrothermal precipitation of a,  $\alpha\text{FeOOH}$ , b,  $\beta\text{FeOOH}$ , c,  $\text{V}_2\text{O}_5 \cdot \text{H}_2\text{O}$  hydrolysis at 313 K.

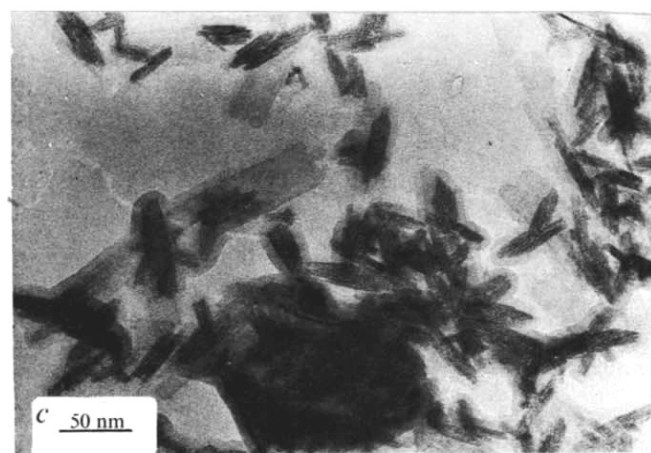
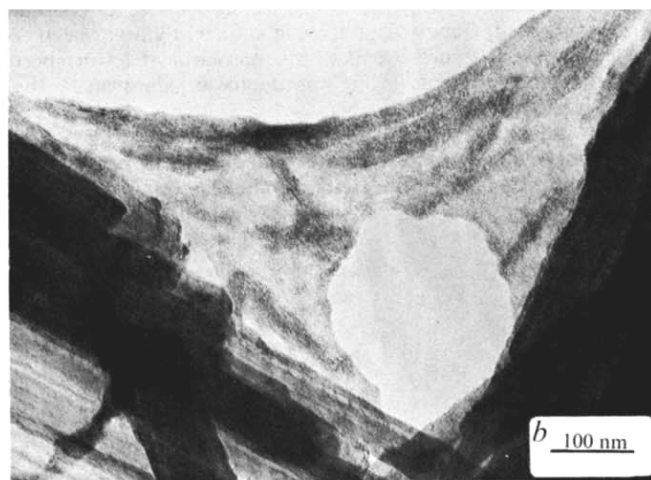
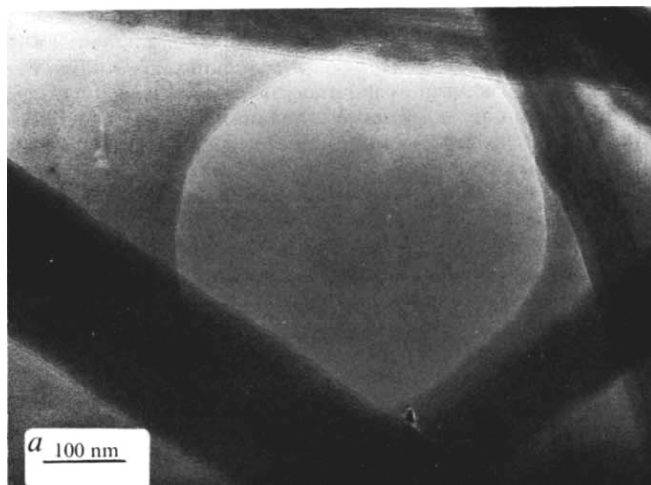


Fig. 2 a, Thin film suspended between asbestos fibres, sample taken at an early stage of film formation from  $\text{FeCl}_3$ , the solution being optically clear. b, Film from  $\text{FeCl}_3$  just before precipitation. The pod-like  $\beta\text{FeOOH}$  crystals are forming on the film the area of which is diminished as the crystals grow. c, Final precipitated product from  $\text{FeCl}_3$  solution— $\beta\text{FeOOH}$ .

and nature of these precipitates arises from the manner of condensation of these films to form a crystalline precipitate.

High resolution electron microscope examinations have been carried out on the aqueous systems  $\text{FeCl}_3 \rightarrow \beta\text{FeOOH}$ ,  $\text{Fe(NO}_3)_3 \rightarrow \alpha\text{FeOOH}$ , and  $\text{VOSO}_4 \rightarrow \text{V}_2\text{O}_5 \cdot \text{H}_2\text{O}$ . Also the morphology of the oxide precipitates from a range of metal oxides has been

surveyed. In all cases the concentration of the solution was 0.01 M and the solution was allowed to stand at, or above, room temperature until precipitation occurred. Samples of the hydrolysis solutions were removed at intervals for electron microscopy and pH measurements. To obtain background-free micrographs and to detect film formation the aqueous samples were sprayed on to a mesh of asbestos fibres supported on an electron microscope specimen grid<sup>1</sup>.

Initially in the  $\text{Fe}(\text{NO}_3)_3$ ,  $\text{FeCl}_3$ , and  $\text{VOSO}_4$  systems there was a marked fall in pH, although at this stage the samples gave no detectable deposit on the asbestos mesh. As the drop in pH diminished, examination by electron microscopy of the samples revealed thin films suspended between the asbestos fibres. The bulk solution remained optically clear during this entire period. We may conclude that the hydrolysis process was largely complete when such films had been formed in solution, since the majority of the theoretically possible fall in pH had taken place. The process of crystallisation had not begun, however, since these films showed no regular structure, nor gave rise to diffraction.

In later samples the film was observed to thicken, in some cases unevenly with regions exhibiting periodic lattice striations. This sequence of events is the same as was observed in the  $\text{ZrOCl}_2 \rightarrow \text{ZrO}_2$  system.

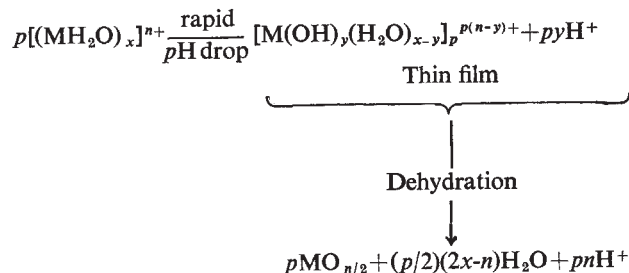
Figure 1 shows the pH/time dependence of the various solutions with the formation of films and precipitates indicated. Figure 2a and b show the films from the  $\text{FeCl}_3$  solution with pod-like structures developing in the film whilst Fig. 2c shows the

had the pod-like shape of  $\beta\text{FeOOH}$  (Fig. 3). Greater  $\text{Cl}^-$  proportions gave pure  $\beta\text{FeOOH}$  crystals and no mixtures of crystal products were observed. During crystal formation there was very little drop in pH. The similarity in appearance between the thin films of polymeric species produced in both the  $\text{Fe}(\text{NO}_3)_3$  and the  $\text{FeCl}_3$  systems indicate that the final crystalline product is determined by the manner in which the anionic counter ion affects the film thickening process.

In these solutions both the  $\text{Cl}^-$  and  $\text{NO}_3^-$  are present in 0.03 M compared to an  $\text{OH}^-$  concentration of  $10^{-11}$  M initially. The  $\text{Cl}^-$  is a stronger complexing agent than  $\text{NO}_3^-$  (ref. 4) but present in a much greater concentration than  $\text{OH}^-$ , therefore, it is reasonable that the thin film would be stabilised as a two-dimensional entity by the presence of  $\text{Cl}^-$  ions, in both of the axial positions of the octahedral  $\text{Fe}^{3+}$  ions, to a greater extent than when  $\text{NO}_3^-$  is a counterion.

The tendency for multilayer formation in the  $\text{NO}_3^-$  system is, therefore, much greater, accounting for the more general thickening of the films, whilst in the  $\text{Cl}^-$  system the film is more likely to crystallise by a rolling up or self condensation mechanism. The nature of the anion and its concentration, is therefore critical to these systems and a strong ligand will suppress or even stop the hydrolysis process.

These results lead to more general conclusions about precipitation mechanisms in metal oxide systems. The first stage is the hydrolysis of the metal ion which then polymerises by way of hydroxide bridges<sup>4</sup> into a two-dimensional film, with a release of hydrogen ions. The crystal formation and precipitation follows this stage with crystal growth being controlled by the anion present, which determines the manner in which the films assume a three-dimensional structure. This growth stage represents the elimination of water from hydroxide bridges to give an oxide structure. These stages may be generally represented as:



where  $x$  is the coordination number of the metal ion  $\text{M}^{n+}$ ,  $y$  has values  $0 \rightarrow x$ , and  $p$  is the number of molecules involved.

The  $\text{OH}^-$  may be replaced by another anion which will be eliminated during the oxide lattice formation, or only partial oxide formation may occur giving rise to oxide hydroxide compounds. The pure oxide can normally be obtained by dehydration.

The pH of the solution drops to approximately 2 at the onset of film formation which would correspond to one hydrogen ion per  $\text{Fe}^{3+}$ . Since four equatorial OH groups would, themselves, be shared between four  $\text{Fe}^{3+}$  ions this agrees with a monomolecular film formation in solution. The final pH of the solution depends upon the acid retention by the final oxide product. These oxides and oxide hydroxides all have a significant ion exchange capacity, and in acid solution there will be surface groups according to the equilibrium:



It is only after prolonged washing that the anion-free precipitate is obtained. For  $\beta\text{FeOOH}$  the ion exchange capacity is  $0.52 \text{ mmol g}^{-1}$  at pH 1.35 indicating approximately one exchangeable site for twenty iron atoms. This would only be possible for a very porous solid, and the regular nature of the pore structure has been reported by Gallacher<sup>5</sup>.

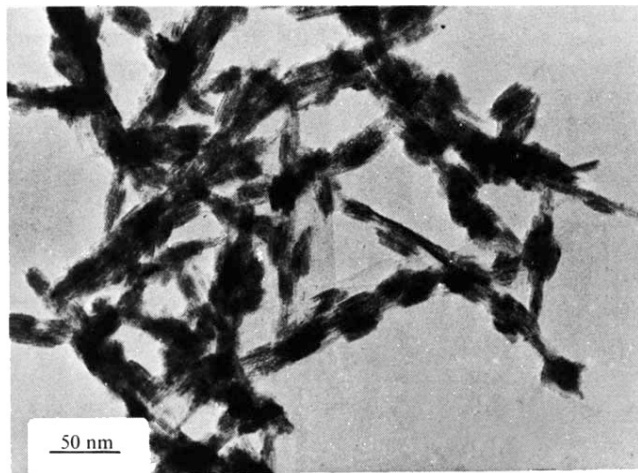


Fig. 3 The final precipitate from a  $\text{FeCl}_3/\text{Fe}(\text{NO}_3)_3$  mixture with a  $\text{Cl}^-/\text{NO}_3^-$  ratio of 0.2. The crystals had the structure of  $\alpha\text{FeOOH}$  but are of similar morphology to  $\beta\text{FeOOH}$ , as in Fig. 2c.

final precipitated product— $\beta\text{FeOOH}$ . The  $\text{VOSO}_4$  and  $\text{Fe}(\text{NO}_3)_3$  systems showed even film thickening and ultimately lath-shaped crystals of  $\text{V}_2\text{O}_5$ ,  $\text{H}_2\text{O}$  and  $\alpha\text{FeOOH}$  were precipitated. Similar lath-shaped crystals were observed as the final precipitates for the systems:  $\text{SnCl}_2$ ,  $\text{SnCl}_4$  and  $\text{AlCl}_3$ .  $\text{NbCl}_5$  and  $\text{TiCl}_4$  gave an irregular crystal and small cubic crystal respectively but in both cases hydrolysis and precipitation occurred immediately the salt was dissolved in water. To investigate the effect of the anion, mixtures of  $\text{Cl}^-$  and  $\text{NO}_3^-$  in the  $\text{Fe}^{3+}$  solutions were hydrolysed, and these gave precipitates of the crystal structure of  $\alpha\text{FeOOH}$  for anion ratios 0–50%  $\text{Cl}^-/\text{NO}_3^-$  but the precipitate



This uniform porosity could be obtained by the rolling up of the thin polymeric films. It is clear that crystallisation does not occur by single atom or molecule addition in any of these systems. The nature of the anion's 'complexing' ability to the metal ion relative to that of water (or OH) will determine whether a layer superimposition occurs, as in  $\alpha\text{FeOOH}$ , or a rolling-up condensation takes place, as in  $\beta\text{FeOOH}$  and  $\text{ZrO}_2$ , giving rise to a regular porosity.

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intermittent aquatic environment, and may best be interpreted as an interdistributary or behind-shore lagoonal facies. The upper part of the sequence was dominated by the influx of fine pyroclastic material now preserved as light coloured, reworked and crossbedded tuffs.

The fossils occur in small pockets within the coarse silt unit over a region of at least 2–3 square miles. Most of the specimens which weather out comprise rhinocerotid or proboscidean postcranial elements. The provisional faunal list is: *Platybelodon kisumuensis*, *Prodeinotherium hobleyi*, *Megalohyrax championi*, *Dicerorhinus* sp., *Listriodon* sp. Creodonta gen. and sp. indeterminate, Crocodylidae gen. and sp. indeterminate, Chelonia gen. and sp. indeterminate.

The presence of medium to small mammals is indicated by enamel and bone fragments which clearly do not belong to the taxa given in the faunal list. As yet, only a small part of the potentially fossiliferous sequence has received even cursory attention. All four identifiable taxa are typical of the early Miocene elsewhere in East Africa and an early Miocene age is confirmed by the radiometric date.

The fauna is not confined to the middle portion of the sequence, for *Prodeinotherium hobleyi* teeth have been collected subsequently from immediately above the basal basalt near the waterhole of Buluk (R. J. G. Savage, personal communication). *Prodeinotherium* teeth have also been found subsequently farther south near the village of Loyengalani where they were associated with a skull and mandible of *Platybelodon kisumuensis* (R. E. Leakey, personal communication). The strati-

## New early Miocene vertebrate locality near Lake Rudolf, Kenya

For the past five years, Plio–Pleistocene sediments cropping out near the north-eastern shore of Lake Rudolf have been under investigation. The lacustrine and fluvial sediments, which extend about 30 km east of the present margin of Lake Rudolf, have yielded a wealth of Plio–Pleistocene palaeontological and archaeological specimens. Provisional accounts of the geology<sup>1,2</sup>, chronostratigraphy<sup>3,4</sup>, archaeology<sup>5,6</sup>, hominids<sup>7–11</sup>, and vertebrate fossils<sup>12</sup> have already been published.

During the summer of 1973, outcrops of Miocene sediments were discovered to the east of the Plio–Pleistocene Lake Rudolf Basin. The Miocene sediments are capped, and in many places covered, by younger igneous rocks that crop out over large portions of the region between Lakes Rudolf and Stephanie. The Plio–Pleistocene sediments are banked against the igneous rocks. Exposure of the Miocene sedimentary sequence seems on present evidence to be limited to a discontinuous belt surrounding the Suregai-Assille basalt plateau. Vertebrate fossils from this sequence were originally discovered during field investigation of the plateau basalts. Several vertebrate sites have been subsequently found on the flank of the plateau around 36° 36'E and 4° 16'N (Fig. 1).

In that region the sediments and associated volcanic rocks are folded into a series of small anticlines and synclines that trend in a NNW–SSE direction. The thickness of the sedimentary sequence is variable but at the vertebrate-bearing localities is about 50 m. There, the sediments lie on top of a deeply weathered and eroded basalt and are in turn capped by a later basalt flow. Potassium–argon age determination of the later basalt has given a date of  $17.3 \pm 1.4$  Myr (ref. 13).

The lower portion of the sedimentary sequence (Fig. 2) comprises a homogeneous and structureless red-brown clay, probably of lacustrine origin. Regression of the lake and its replacement by fluvial and flood plain environments accounted for the subsequent superposition of a coarse silt sequence intercalated with sandstones, clays and fine silts. The most prolific vertebrate-bearing site occurred in a silt bed rich in root casts, algal structures and burrows. The bed is of limited lateral extent, seems to have accumulated in a shallow or

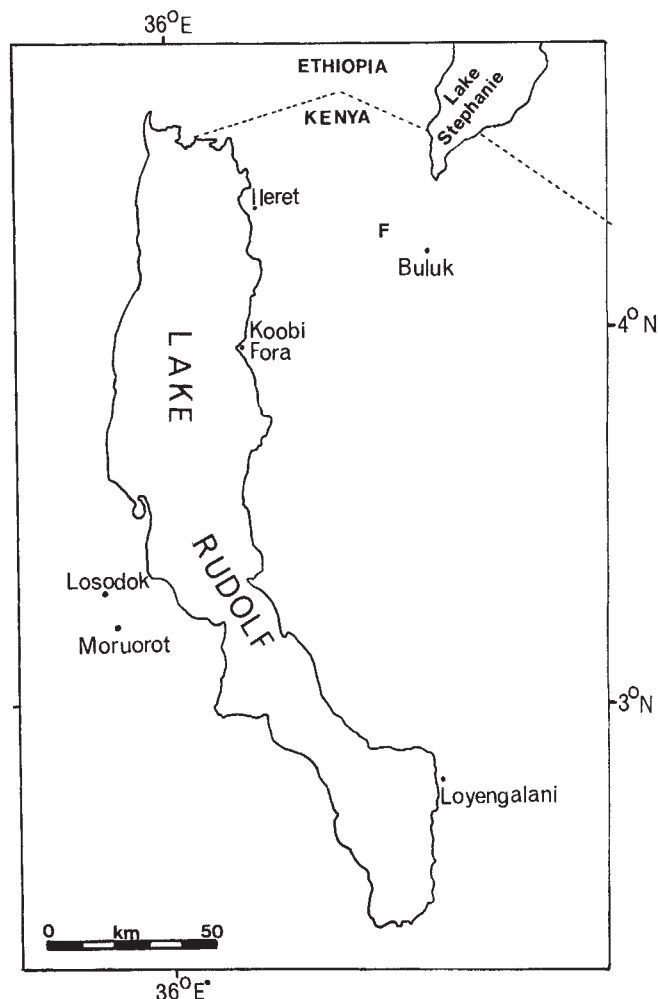


Fig. 1 Location of new Miocene locality (F).

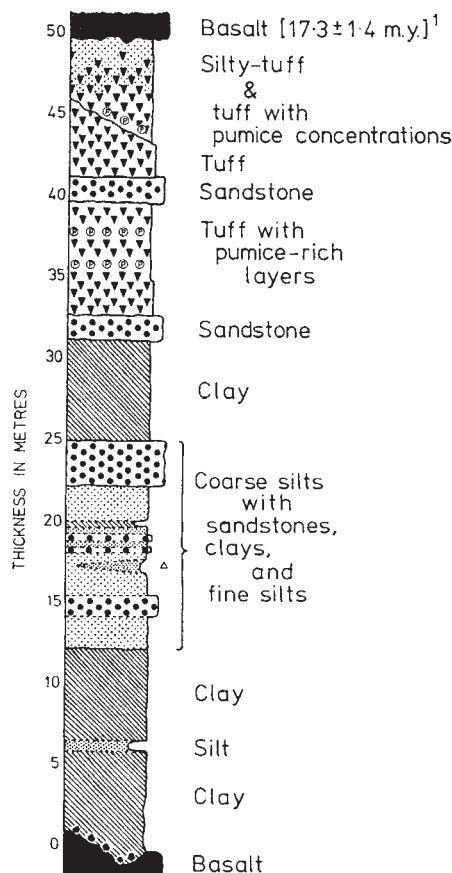


Fig. 2 Stratigraphical section at new early Miocene locality.  $\Delta$ , Position of main vertebrate localities.

graphical relationship between the latter region and the Suregai-Assille plateau is not yet clear.

A currently more prolific early Miocene fauna has been recorded from sites of more limited lateral extent on the western side of Lake Rudolf<sup>14,15</sup>. The areal extent of the new localities on the eastern side of the lake awaits determination but is likely to prove larger than any East African site known to yield early Miocene vertebrate fossils. The new localities extend the northern limit of sub-Saharan Miocene faunas from the East African Rift System and provide complementary potential to the East Rudolf Plio-Pleistocene sediments for providing evidence of hominid origins and early evolution.

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## Discrimination in the assimilation of *n*-alkanes in fish

VERY large quantities of hydrocarbons have been and continue to be introduced into the seas and oceans by the various activities of man<sup>1,2</sup> but estimates of this input and comparisons with biogenically-derived material or seepage from fossil hydrocarbon deposits are difficult to make. An assessment of the general effects of non-biogenic hydrocarbons on the marine ecosystem is equally difficult.

In the analyses<sup>3</sup> we have carried out on flesh and livers from wild codling caught round the coast of the British Isles the *n*-alkane patterns observed fell into two general types (Fig. 1). The flesh patterns had a relatively smooth profile with a minimum around hexadecane (C16), a maximum at C26–C28 and no marked odd carbon atom predominance. The liver patterns, however, showed a marked odd carbon atom predominance, a maximum at C29–C31 and, as in the flesh, a minimum in the C16 region. These profiles bear little or no resemblance to the various alkane patterns of crude oils or indeed to most hydrocarbon products. Superficially, this suggests that the alkanes were not derived from the non-biogenic hydrocarbon input to the marine environment, but our laboratory-based studies with cod (*Gadus morhua*) suggest that this is not necessarily the case.

Cod liver oil capsules (containing 49 mg liver oil and 1 mg topped Kuwait crude oil) disguised in the normal squid diet were fed to codling (weight approximately 500 g) at a concentration of 1 mg crude oil d<sup>-1</sup> for 175 d. The same diet without the crude oil was fed concurrently to a control group. During the subsequent 109 d period the capsules were withdrawn from the diet. The *n*-alkanes content and pattern in the livers after 6 months feeding with oil showed remarkable changes (Fig. 2) in that the profile of the livers of the crude oil-fed fish lost the odd carbon atom alkane predominance and assumed a profile similar to that of the flesh (Fig. 1). The relative changes in the amount of *n*-alkanes are given in Table 1.

Figure 3 shows a discriminatory uptake of alkanes by the liver. Thus, the alkanes around C16 do not seem to be retained to any great extent, but as the chain length increases to about C26 the relative amount retained rises to a maximum. It is not possible to state from the results of this experiment whether these differences in the retention of the *n*-alkanes were due to a discriminatory absorption mechanism by the liver and/or the digestive system, and/or a preferential metabolism and/or excretion of the

Table 1 Relative changes in the amount of *n*-alkanes

|         | $\sum_{C15}^{C33}$ <i>n</i> -alkanes ( $\mu\text{g g}^{-1}$ tissue) |         |        |         |       |        |
|---------|---------------------------------------------------------------------|---------|--------|---------|-------|--------|
|         | 0 d                                                                 |         | 175 d  |         | 284 d |        |
| Codling | Liver*                                                              | Muscle* | Liver* | Muscle* | Liver | Muscle |
| Control | 3.4                                                                 | 0.2     | 4.9    | 0.2     | 3.4†  | 0.1†   |
| Treated | —                                                                   | —       | 29.9   | 0.2     | 13.3‡ | 0.2‡   |

\* Mean of three fish.

† Mean of four fish.

‡ Mean of five fish.



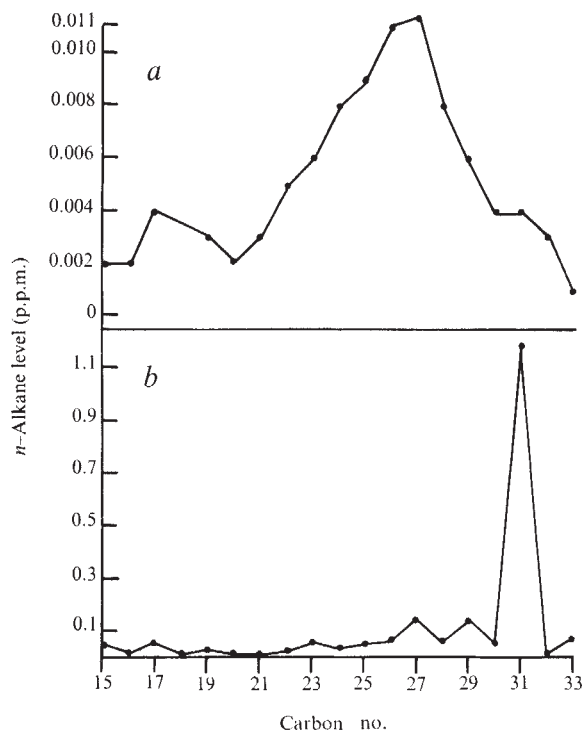


Fig. 1 Distribution of *n*-alkanes in wild codling flesh (a) and liver (b).

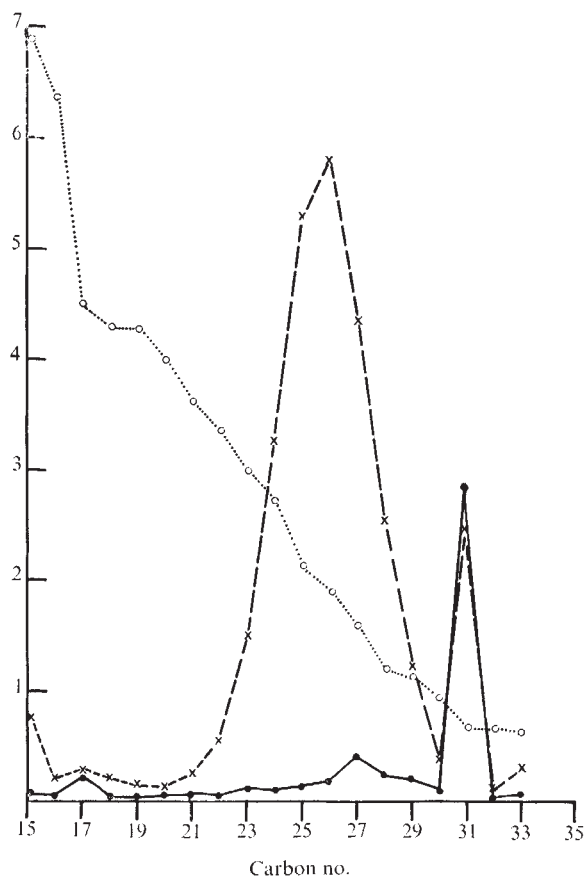


Fig. 2 Distribution of *n*-alkanes.  $\times$ , livers from codling fed crude oil for 6 months;  $\bullet$ , control livers (both in p.p.m.) and,  $\circ$ , the crude oil used in the experiment (in  $\mu\text{g}$  alkanes per 100 mg crude oil). The liver results are from a mean of three fish.

shorter chain hydrocarbons. After the 6-month period following cessation of crude oil feeding, the alkanes in the liver had fallen considerably (Table 1) but the residual *n*-alkane fraction retained essentially the same oil-induced profile.

Additional experiments carried out by feeding cod with single doses of  $2 \mu\text{Ci } 1\text{-}^{14}\text{C}$  hexadecane (disguised in the normal squid diet) showed that only 0.4% of the activity was recovered from the liver. Analysis of the active material by thin layer chromatography showed that it was entirely unchanged hexadecane. This seems to rule out a high metabolic conversion of hexadecane in the liver and lends some support to the presence of a mechanism in cod which discriminates between the uptake of individual *n*-alkanes in the chain length range C15–C33. This has obvious implications in considering the assimilation of hydrocarbons whether of recent biogenic or fossil fuel origin and particularly so if conclusions are drawn for the alkanes as a whole on the basis of experiments with a specific compound.

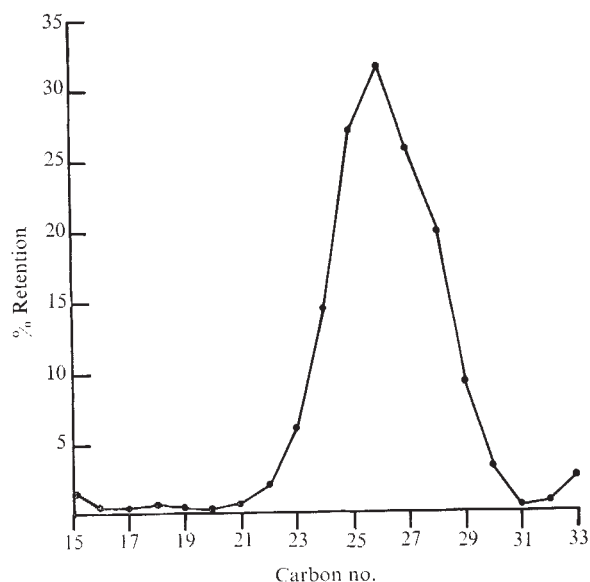


Fig. 3 Percentage retention of *n*-alkanes by the codling liver after 6 months feeding with oil as a function of carbon number.

In the cod, the liver is probably the centre of lipid metabolism and may well exercise some function of control in the deposition of alkanes in the tissues even though there is such a striking difference between the profiles of liver and flesh in normal fish. The apparent discrimination indicated by the liver analyses in the presence of a specific alkane array in the diet demonstrates clearly that, in this species, it is unlikely that a simple comparison of tissue analyses with those of the extraneous hydrocarbon source will suffice to identify the source of the tissue alkanes.

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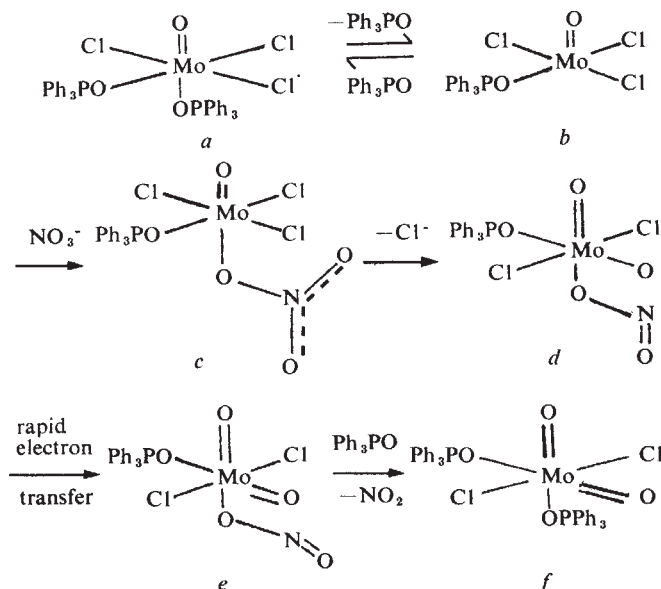
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## Possible model reactions for the nitrate reductases

NITRATE is the major source of nitrogen for most green plants and fungi and may also be used by certain micro-organisms as a terminal electron acceptor in place of oxygen under anaerobic conditions. The enzymes responsible for the first step in nitrate assimilation and for nitrate respiration are the nitrate reductases, both these processes involving the conversion of nitrate to nitrite. The nitrate reductases require molybdenum both for their formation and activity<sup>1,2</sup>. Chemical tests have indicated that during the enzymatic reduction of nitrate to nitrite, the oxidation state change undergone by the molybdenum present in the enzyme from *Neurospora crassa* involves Mo(V) and Mo(VI)<sup>3</sup> and ESR spectroscopic studies have strongly suggested that the nitrate is directly bound to the Mo(V) centre of the enzyme from *Micrococcus denitrificans* before reduction<sup>4</sup>. Therefore we have investigated whether simple molybdenum(V) complexes are able to reduce nitrate to nitrite. This has been attempted previously and either no reduction of nitrate was detected<sup>5,6</sup> or reduction occurred to yield nitric oxide as the major product<sup>6</sup>. Similarly, molybdenum-catalysed reductions of nitrate have produced at the most only traces of nitrite<sup>7-9</sup>.

We report here that oxo-molybdenum(V) complexes such as  $[\text{MoOCl}_2\text{L}_2]$ ,  $[\text{MoOCl}_2\text{L}]^-$  (where  $\text{L} = \text{Ph}_3\text{PO}$  or  $(\text{Me}_2\text{N})_3\text{PO}$ ) or  $[\text{MoOCl}_5]^{2-}$  in dichloromethane solution at room temperature, will rapidly and quantitatively convert nitrate to nitrogen(IV) oxide and slowly and quantitatively reduce nitrogen(IV) oxide to nitrogen(III), probably as  $\text{NO}^+$ . The mechanism of the reaction between an excess ( $\geq 6:1$ ) of  $\text{Et}_4\text{NNO}_3$  and  $[\text{MoOCl}_2(\text{OPPh}_3)_2]$  under the above conditions has been investigated using stopped-flow kinetic techniques. The results obtained are consistent with the following mechanism:



The nitrogen(IV) oxide has been removed from a 1:1 reaction mixture under reduced pressure, collected and identified by ultraviolet and infrared spectroscopy. The complex  $[\text{MoO}_2\text{Cl}_2(\text{OPPh}_3)_2]$  has been obtained from solutions containing nitrate and molybdenum(V)  $\leq 2:1$ ; for the higher nitrate ratios used in the kinetic studies, the redox reaction appears to be followed by nitrate substitution at the molybdenum(VI) centre.

The labilisation of the ligand *trans* to an oxo group is a

well known effect<sup>10</sup> and for  $[\text{MoOCl}_2(\text{OPPh}_3)_2]$  this results in the formation of the five-coordinate intermediate *b* which possesses a site available for unidentate coordination of a nitrate group to produce *c*. A preference for the coordination of nitrate as a bidentate ligand has been noted<sup>11</sup> and *d* is apparently formed by chelation which involves the displacement of a chloride ion. The geometry of *d* is ideal for a rapid intramolecular electron transfer from the molybdenum(V) centre to the nitrate group (Fig. 1). The typical electronic ground state for octahedral oxo-molybdenum(V) complexes seems (ref. 12 and C. D. Garner, P. Lambert, and F. E. Mabbs<sup>1</sup>, unpublished) to be  $4d_{xy}$ .

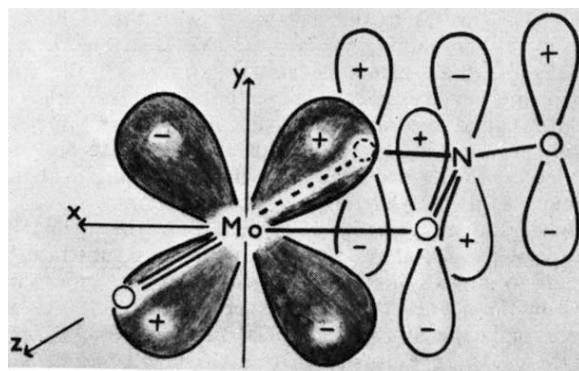


Fig. 1 Coordination of nitrate group to oxo-molybdenum(V) centre.

Nitrato complexes which contain transition metal ions in high oxidation states are powerful oxidants<sup>11</sup> and *ab initio* molecular orbital calculations (I. H. Hillier, private communication) suggest that this is a result of the considerable lowering in the energy of the  $\pi^*$  orbital of the nitrate group on coordination. A rapid electron transfer from the molybdenum(V) centre to the nitrato group requires<sup>13,14</sup> that the metal's  $4d_{xy}$  orbital overlaps to some extent with the  $\pi^*$  orbital of the nitrato group. Such overlap is only possible when the nitrato group has one and only one oxygen atom coordinated *cis* to the oxo group. Following the redox reaction, the electron transfer  $\pi^* \rightarrow \sigma^*$  within the nitrato group and the (slight) atomic rearrangements necessary would be expected to proceed rapidly to produce *e*, involving the expected<sup>10</sup> *cis*- $\text{Mo}^{\text{VI}}\text{O}_2$  unit and nitrogen(IV) oxide. As the latter has no known tendency to form complexes it is presumably readily displaced by the free triphenylphosphine oxide. At initial molybdenum(V) and nitrate concentrations of around  $1.5 \times 10^{-4}$  and  $2.5 \times 10^{-3}$  mol  $\text{l}^{-1}$ , respectively, this reaction sequence is completed in  $< 15$  s at  $25^\circ\text{C}$ . The addition of water to the reaction mixture at this stage results in the disproportionation of the nitrogen(IV) oxide into nitrate and nitrite<sup>8,9</sup>, the amount of the latter being measured colorimetrically<sup>15</sup>.

The results suggest some criteria which may be important concerning the nature of the molybdenum(V) centre in the nitrate reductases. Coordination of nitrate to the metal centre could be important in lowering the activation energy for electron transfer to this group. A non-aqueous environment would favour this coordination<sup>11</sup> and therefore we suggest that the molybdenum centre of the nitrate reductases is in a hydrophobic region of the protein (which probably plays an important role in introducing the substrate to the coordination sphere of the metal). An enzymatic redox reaction between molybdenum(V) and nitrate producing molybdenum(VI) and nitrogen(IV) oxide has two main attractions: not only would the reduced species be readily displaced from the metal centre but also, once



in contact with water out of the vicinity of the metal centre, it would yield nitrite and nitrate ions, thus giving the appearance that the enzyme is actually converting nitrate to nitrite directly. The detection of nitrogen(IV) oxide in the natural systems will be difficult. ESR spectroscopy could be used, although the signal obtained from this free radical in solution can be broad and ill-defined<sup>18</sup>. The presence of an oxo group on the molybdenum(V) centre would be expected on chemical grounds<sup>10</sup> and this group would probably dominate the reaction pathway in two respects. First, in aiding initial substrate binding *trans* to the oxo group<sup>17</sup> and second in controlling the electronic structure at the metal. Facile electron transfer from an oxo-molybdenum(V) centre to a nitrate group will only occur if the latter is able to coordinate by way of one oxygen atom at a site *cis* to the oxo group with the plane of the NO<sub>3</sub> moiety containing the Mo=O axis (Fig. 1). Therefore, we suggest that, after the initial binding of the nitrate group, the enzymes must ensure that either suitable chelation or ligand rearrangement of this substrate is possible to enable one (and only one) of the oxygen atoms to be located *cis* to the oxo group. As there is strong evidence in favour of a molybdenum cofactor common to all the molybdenum enzymes<sup>18</sup> we suggest that the hydrophobic nature of the metal site and the facility for substrate binding *cis* to an oxo-molybdenum group may be important to the function of all the enzymes of this class.

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## Lack of effect of *Avena sativa* on cigarette smoking

AFTER his conclusion that an alcoholic extract of *Avena sativa* (the common oat) reduced craving for and consumption of cigarettes, Anand<sup>1</sup> recommended further investigation of its significance and role. We attempted the former by repeating his study in a situation less likely to prejudice participants than a chest ward and the latter by using a study design which would have cast light on the role of the treatment in altering smoking if such an effect had been evident.

The botanical state of his starting material was variously described as "selected just before harvest"<sup>1</sup> and presumably yellow, and "ripe and green"<sup>2,3</sup>. Thus contents of the putative active ingredient could differ. We therefore studied both a green plant with full sized ears and a ripe plant dug up one week before the main harvesting. Ethanol extracts of the plant including its roots were made exactly as described by Anand<sup>1</sup>. In preliminary blind studies the extract was nearly always distinguished from dummy despite the resources of a pharmaceutical laboratory. Therefore, two differently tasting liquids though approximating the musty quality of the oat infusion were prepared with an identical alcohol content. All three were coloured deep red. Subjects knew two dummies were included in varying order so they could not conclude from a change in taste that a period of test material had begun.

Employees of The Wellcome Foundation, Crewe, volunteered in response to a notice requesting help from smokers who would not object if their smoking habits altered while testing the effect of a new drug on smoking. No direct appeal was made to those anxious to stop smoking. At the end of the study a reward of 50p was offered. Smokers bought their own cigarettes.

Subjects had diary sheets and recorded their daily cigarette consumption by joining up any two dots randomly from an array of dots for each cigarette, thereby hindering a too ready comparison of their daily consumption. They also recorded the doses of extract taken each day, a subjective enjoyment rating of the day's cigarettes (better, same, less, none), any unwanted symptoms or effects as, for example, those brought on by a cold and their opinion as to whether they had received dummy or oat extract. They knew no treatment would last for less than one week and that the first would be a dummy to provide a run-in period. Diary pages were collected from the subjects separately each week and the next week's liquid issued in exchange for the used bottle. Forty-three subjects participated in the first study and eighteen of them continued into the second study.

Treatments were of 1 ml of appropriate ethanolic liquid diluted with 4 ml of water for palatability and taken four times daily during waking hours. They were presented as shown in Table 1.

Table 1 Treatment schedules for smokers

| Study 1<br>week    | Extract immature oats (EIO) v dummy (D) |         |         |         |
|--------------------|-----------------------------------------|---------|---------|---------|
|                    | Group 1                                 | Group 2 | Group 3 | Group 4 |
| 1                  | D1                                      | D2      | D2      | D1      |
| 2                  | D2                                      | D1      | D1      | D2      |
| 3-6                | EIO                                     | EIO     | D2      | D1      |
| 7-10               | D1                                      | D2      | EIO     | EIO     |
| 11-12              | No treatment                            |         |         |         |
| No. of<br>subjects | 7                                       | 14      | 7       | 15      |

Volunteers had to decide whether they had taken oats or dummy infusion. Statistical analysis suggested that their decisions were guesses rather than detections of the oat preparation. Any persistent effects would have been observed in the period following the oats administration. The volunteers expected to learn at the end of the tenth week in which period

they took the test liquid. Actually all were misinformed and only correctly informed at the end of the twelfth week. If any real effect had operated by psychological rather than pharmacological means it might have diminished in volunteers of groups 3 and 4 during weeks 11 and 12.

Subjects' cigarette consumptions at a rate different from that of groups 1 and 2 during weeks 7 and 8, were compared for each week and period of the trial, allowing for different order of administration of preparations, by means of analysis of variance. The mean daily cigarette consumptions for each period in study 1 (43 subjects) were: run in (weeks 1 and 2) 23.5; EIO (weeks 3–6 or 7–10) 24.4; dummy (weeks 7–10 or 3–6) 24.0; run out (weeks 11 and 12) 25.4 (36 subjects). Individual subjects' mean daily consumption varied between 12 and 42 per day in the run in period, individual extreme consumptions being 6 and 54. Twenty-three subjects' mean daily consumptions were higher in the EIO period than the dummy period; fifteen consumed less in the EIO period and five did not change. The two largest individual changes in daily consumption between periods on EIO and dummy were respectively a rise from 21 to 27 (average 21 on run in) and a fall from 32 to 25 (26 on run in).

The study finished just before a Christmas holiday when unusual social factors affect smoking habits. The study of the mature oats was therefore postponed. As the volunteers were no longer naïve (nor could any others from the same community be expected to be) dummies were not given during the run in periods. Instead, one-half took immature oats infusion over weeks 2 to 4 while the other took mature oats and in weeks 5 to 7 each group took the alternative. No significant differences between EIO and EMO were evident. Mean daily cigarette consumptions in study 2 (18 subjects) were run in 25.6, EIO 24.7 (14 subjects) and EMO 24.9. Four subjects stopped smoking after completing the second study because the 'budget' raised the cost of cigarettes. Regrettably we conclude that the views of our compatriot, Dr Samuel Johnson, on oats would not have been modified by our study, though by its lucky timing his views on excise might have been.

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## Marijuana metabolites measured by a radioimmune technique

THE recent surge of interest in the pharmacology of cannabinoids has created a need for a rapid assay for  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC) and its metabolites in body fluids. A sensitive immune technique has proved elusive, and quantitation requires a multistep chromatographic and mass spectroscopic analysis<sup>1</sup>. Gas liquid chromatography with electron capture detection has also been used<sup>2,3</sup>. We describe here a simple rapid radio-immune assay for  $\Delta^9$ -THC utilising goat antiserum obtained as previously described<sup>4</sup>.

A standard antibody-binding curve was obtained as follows: 4.5 ng (5,000 c.p.m.) of  $^3\text{H}$ - $\Delta^9$ -THC (specific activity 465 mCi/mmol<sup>-1</sup>, more than 98% pure, New England Nuclear Corp.) was incubated for 2 h at 4° C with increasing dilutions of antiserum (0.3 ml). The latter were made up with phosphate-buffered saline (PBS), 0.1 M in phosphate, pH 7.0 in 0.1% human  $\gamma$  globulin. After this initial incubation, 1 ml of a cold

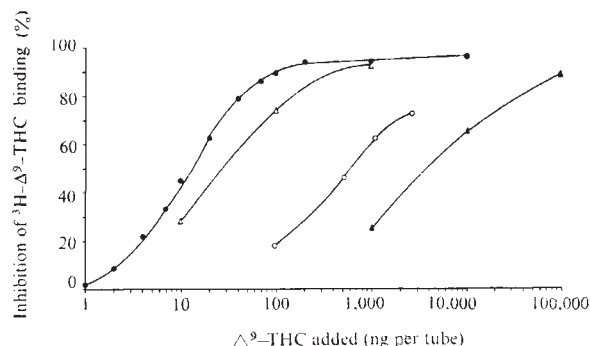


Fig. 1 Inhibition of  $^3\text{H}$ - $\Delta^9$ -THC binding to antibody by increasing amounts of unlabelled THC metabolites. ●,  $\Delta^9$ -THC; △, 11-hydroxy- $\Delta^9$ -THC; ○, 11-nor-9-carboxy- $\Delta^9$ -THC; ▲, cannabidiol.

dextran charcoal suspension (0.25% dextran–2.5% activated charcoal in PBS), was added and incubation continued for 15 min. The tubes were then centrifuged at 3,000 r.p.m. for 15 min at 4° C and the supernatant (1 ml) was counted in a Nuclear Chicago liquid scintillation spectrometer (70% tritium counting efficiency) with Aquasol (New England Nuclear Corp.), as the scintillation fluid. Binding of  $^3\text{H}$ - $\Delta^9$ -THC by normal goat serum under the foregoing conditions was slightly above background. A maximum of 50% of marker was bound by a 1/60 early antiserum dilution. The latter serum dilution was used in the inhibition experiments described below.

To ascertain the specificity of the antiserum, various amounts of cold  $\Delta^9$ -THC, 11-hydroxy- $\Delta^9$ -THC, 11-nor-9-carboxy- $\Delta^9$ -THC and cannabidiol were added to a 1/60 dilution of antiserum 30 min before addition of 4.5 ng  $^3\text{H}$ - $\Delta^9$ -THC. The sensitivity of the inhibition assay was 1.2 ng per tube at a 95% confidence limit. A sample inhibition experiment at 50% binding is plotted in Fig. 1 and percentage cross reactivities are tabulated in Table 1.

Table 1 Cross reactions of cannabinoids with THC antiserum

|                      | % Cross reaction |
|----------------------|------------------|
| $\Delta^9$ -THC      | 100              |
| 11-Hydroxy-THC       | 47               |
| 11-Nor-9-carboxy-THC | 2.6              |
| Cannabidiol          | 0.5              |

\*At 50% inhibition

The association constant for the antiserum was determined graphically from a Scatchard plot. In the range of dilutions tested, crude antiserum bound THC at  $K_a = 4.0 \times 10^8$  M indicating a potential sensitivity of 300 pg per tube.

After a preliminary adsorption with charcoal<sup>5</sup>, samples of male plasma treated with known quantities of  $\Delta^9$ -THC (50–700 ng ml<sup>-1</sup>) were measured. Samples of 1 ml were shaken with heptane ethanol (100:1.5), the extracts were dried and the residue was dissolved in 0.5 ml of 50% ethanol. This solution (50  $\mu$ l) was used in inhibition assays. Ideally the assays should have shown 5, 10, 20, 50 and 70  $\mu$ g per tube, respectively. Their deviation from these points on the standard curve (Fig. 2) was small and within the limits of experimental error.

Plasma samples were obtained from chronic users of marijuana who were part of a controlled marijuana research project and had used no other drug for at least 10 d previously. Blood was drawn 15 min after they had smoked a marijuana cigarette (0.9 g) containing 2.1% (18.9 mg)  $\Delta^9$ -THC. Plasma was separated by centrifugation and stored frozen at –10° C. These samples (1–3 ml) were extracted with heptane–ethanol. Aliquots (0.25 ml) of reconstituted extract were used in the radioimmune assay system already described.

In each of six plasma samples  $\Delta^9$ -THC was detected and quantitated 30 min before and 15 min after THC use. Pre-intoxication levels were 60–100 ng ml<sup>-1</sup>. Postintoxication levels



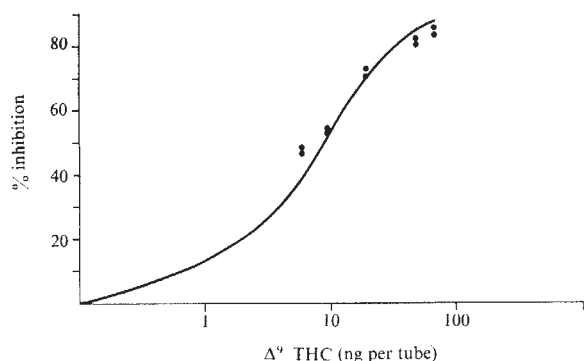


Fig. 2 Percentage inhibition of  $^3\text{H}$ - $\Delta^8$ -THC-azoTHC binding by (—) various amounts of cold  $\Delta^8$ -THC added from a stock reagent vial, and (:) various amounts of cold  $\Delta^8$ -THC extracted from serum samples after treatment with  $\Delta^8$ -THC. Tests were done in duplicate.

were 200–250 ng ml<sup>-1</sup>. This represents 0.032% of total THC in the standardised cigarette. A significant portion of the remainder was presumed to have been exhaled. The amount of THC distributed in other body compartments is an important consideration but not of concern here.

Immunisation with  $\Delta^8$ -THC protein conjugates and testing of antibodies with plasma samples have been hampered by the relative insolubility and nonspecific binding of THC to serum protein. This binding can result in very high blanks and thus interfere with inhibition and assay interpretation. This was overcome in our early studies involving fluorescence by minimal fluorescence perturbation with nonspecific THC-protein binding and in recent experiments involving radioimmune methods through manipulation of the charcoal suspension.

Our antiserum was characterised by a low cross reactivity with closely related THC metabolites and other cannabinoids, the only exception being 11-hydroxy- $\Delta^8$ -THC, now considered an insignificant contributor (paper presented by M. Wall at International Congress of Pharmaceutical Sciences, Sweden, 1973). The relative insolubility of 11-nor-9-carboxy- $\Delta^8$ -THC in the extraction solvent (heptane-ethanol) and its low cross reactivity with  $\Delta^8$ -THC makes its contribution negligible.

Although the sensitivity of our method is limited to 25–50 ng ml<sup>-1</sup> (4.5 ng of  $^3\text{H}$ - $\Delta^8$ -THC is required to provide 2,000 c.p.m. per sample tube), it can be used to estimate cannabinoids in chronic marijuana users. We are developing markers with much higher specific activities (100–200 Ci mmol<sup>-1</sup>) to enhance sensitivity into the pg ml<sup>-1</sup> range, for clearance studies and investigation of occasional abusers.

For body fluid determinations to be made several hours or more after exposure to marijuana, it would seem desirable to detect 11-nor-9-carboxy-THC<sup>6</sup>. Therefore we are preparing antibodies which are directed separately to the latter metabolite\*. Thus body fluid and tissue contents of each metabolite should more easily be correlated with psychoactive effects than was previously possible.

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\* Note added in proof: We now have a simple radio-immune assay specifically towards this important metabolite.

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## Cortical potentials evoked by finger displacement in man

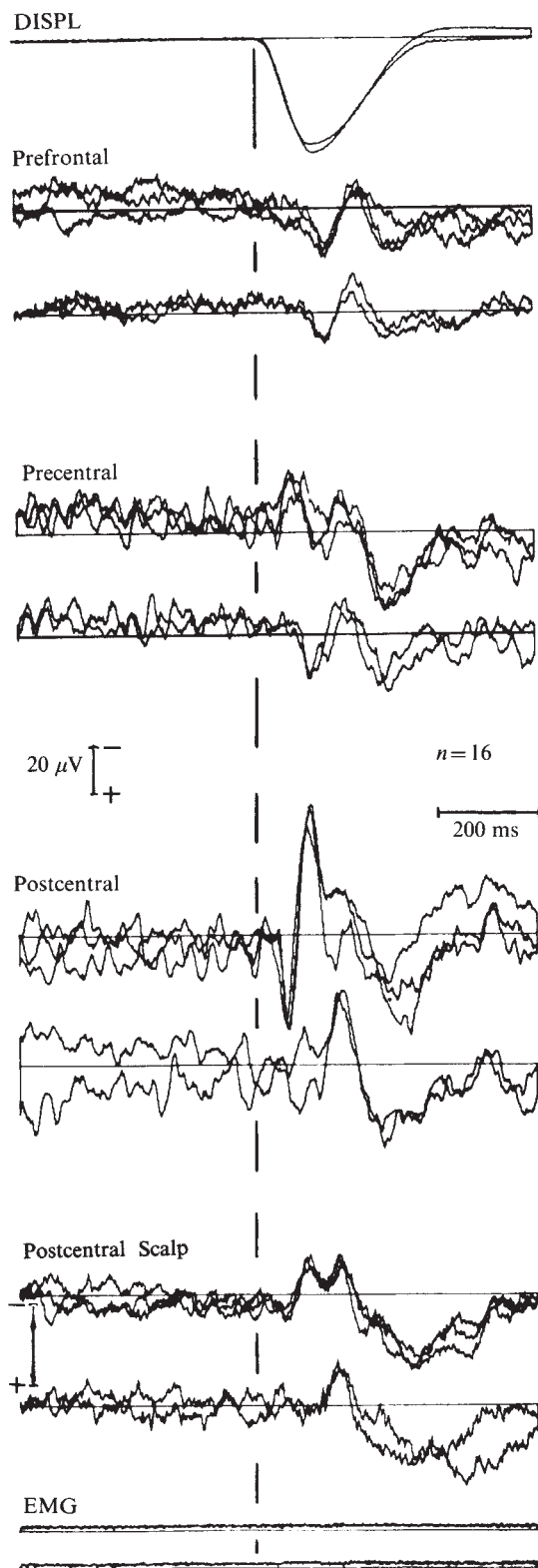
THE neuropsychophysiology underlying the sense and change of limb position in animals and man is a centre of enquiry by many disciplines ranging from medicine and physiology to psychology and sociology<sup>1</sup>. Consistent changes in the electrocorticogram (ECoG) following change of position have, however, not yet been reported in man, although recordings of unit activity during neurosurgical operations have demonstrated neuronal activity in cortex<sup>2</sup> and subcortical structures<sup>3–5</sup>. Limited data from scalp recordings after passive movement have also been reported<sup>6,7</sup>.

Electrical changes, time-locked to movement of the index finger, have now been recorded from twelve subjects using cortical and scalp electrodes. The cortical recordings have been obtained from chronically implanted subdural gold wire electrodes in seven subjects selected for treatment by multifocal leukoagulation<sup>8,9</sup>. Simultaneous cortical and scalp recordings were taken from these patients. Scalp recordings have been obtained from five normal subjects. The position of the cortical electrodes was determined by X-ray photography and by the reversal across the central fissure of the early negative component of the somatosensory-evoked responses after contralateral median nerve stimulation<sup>10–12</sup>. Precise localisation of the electrodes was not always possible and they have been grouped in three areas: (1) postcentral, immediately posterior, and (2) precentral, immediately anterior, to the central sulcus; and (3) prefrontal, close to the tip of the frontal lobe. All electrodes were in the right hemisphere about 5 cm lateral to the sagittal plane. The scalp electrodes were placed 2 cm above the nasion to monitor eye movements, at the vertex, and over the pre- and postcentral cortical areas of both hemispheres.

Displacement of the left or right index finger by about 45° around the metacarpophalangeal joint was by a mechanical arrangement which permitted unobstructed return of the finger to the resting position. The displacement was monitored using a movement transducer. The differentiated and grossly amplified output of this transducer was used to trigger the PDP-12 computer used for multichannel data collection of the electrical brain activity for 1 s before and 1 s after the displacement. The electromyogram (EMG) from the arm flexors and extensors were also recorded. Time constant and upper frequency response of the electroencephalogram were 1.2 s and 700 Hz, respectively.

In all subjects, cortical and scalp recordings showed consistent responses after displacement. Figure 1 shows the responses recorded from three cortical and one scalp electrode to contralateral and ipsilateral displacement. To contralateral displacement the pre- and postcentral cortical areas showed an initial positivity with a delay of  $34 \pm 6$  ms to peak and  $42 \pm 4$  ms, respectively. The amplitudes ranged from 5 to 25  $\mu\text{V}$  across subjects and were usually larger in postcentral areas. This positivity was followed by a negative wave with a peak latency of  $68 \pm 15$  ms for precentral and  $97 \pm 20$  ms for postcentral areas, its amplitude ranging from 30 to 50  $\mu\text{V}$  across subjects. The response continued for 400 ms with a number of positive and negative components. These late components, although consistent for an individual, were variable across subjects. In the prefrontal

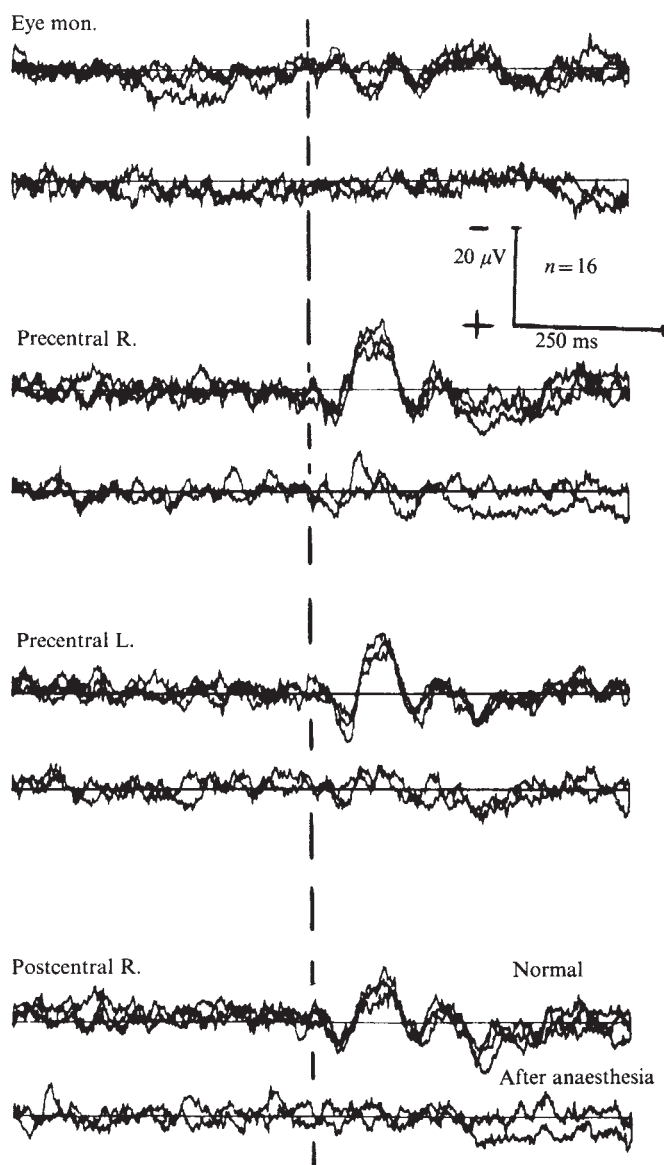




**Fig. 1** Superimposed average cortical and scalp responses following externally paced left (upper trace of every pair) and right (lower trace of every pair) index finger displacement (DISPL). Interrupted vertical line shows the trigger point. Both cortical and scalp recordings from the right hemisphere with electrodes at prefrontal, precentral and postcentral cortices. The electromyogram (EMG) of the arm flexors does not change after displacement. Note the consistency in waveform for the same area under similar stimulus condition, the differences in waveform for different areas simultaneously registered and the different response of the same area, except prefrontal, to contralateral and ipsilateral displacement. Cortical electrodes referred to average of 60 electrodes in frontal lobe. Scalp electrodes referred to commoned electrodes on each mastoid process.

areas the responses to contralateral displacement started later than 100 ms and were similar to those evoked by ipsilateral movement. After ipsilateral finger movement, the pre- and postcentral areas gave different responses from those after contralateral displacement. The ipsilateral responses started with a positive deflection with a latency of 60 ms or more. Comparisons within subjects, of the total response to contralateral displacement from prefrontal, precentral and postcentral areas, showed different waveforms. Cross-correlations of responses recorded from electrodes within any one of these areas were high (about 0.85) but cross-correlations between responses from different areas were low (about 0.25).

Flexion or extension of the limb did not change the configuration or the spatial distribution of the displacement-evoked potentials. This and the absence of EMG activity



**Fig. 2** Superimposed average, scalp-recorded, responses following externally paced left index finger displacement. Interrupted vertical line shows the trigger point. Eye mon., activity at an electrode 2 cm above the nasion for monitoring eye movements. Electrodes corresponding to precentral and postcentral cortical areas of the left or right hemisphere are marked L or R. Upper trace of every pair, normal activity of the indicated location before, during and after displacement. Lower traces, activity of the same locations to similar displacement 45–55 min after anaesthesia of the hand with anoxia (AN). Note the loss of the responses after anaesthesia.

during the displacement indicate that muscle afferents contribute very little, if at all, to this response. To test this hypothesis anaesthesia of the joint has been imposed through ischaemic anoxia in normal volunteers, as previously described<sup>13</sup>. For 10 min the blood circulation in arm and hand was stopped by applying pressure with the cuff of a manometer just above the elbow joint. A second manometer encircled the wrist and was inflated and the pressure above the elbow relieved, thus permitting free blood circulation for the arm muscles. Scalp responses evoked between 45 and 55 min after anoxia are compared with the pre-anoxic responses in Fig. 2. The response is greatly reduced although the muscles of the arm and their sense organs are in normal conditions. We conclude that muscle afferents do not contribute to the displacement-evoked responses which most probably reflect activation of joint afferents. Activation of these afferents evokes different electrical changes in pre-frontal, precentral and postcentral cortical areas. This differential representation in the cortical level of the input activated by displacement, as well as the timing of the various components of the response, offer an objective index in understanding the brain mechanisms of position sense in normal man and in patients.

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## Visualisation of micropore structure in human dental enamel

DENTAL enamel has an internal void volume of 0.2-5%<sup>1-3</sup>. The direct observation of pores in enamel, however, has been hindered by inadequate specimen preparation techniques. The harsh chemical treatment necessary to partially demineralise enamel prior to embedding and sectioning, and the chatter and shattering effects which result from cutting hard tissues, create artefactual spaces in enamel which make it virtually impossible to identify and define any natural spaces present. Attempts to cut enamel which has not been demineralised produce even greater shatter and destruction of the natural structure.



**Fig. 1** Transmission electron micrograph of longitudinal section of ion beam thinned human dental enamel showing the long lath structure of apatite crystals with electron lucent channels or pores between them. Ion beam thinning may remove organic material faster than the mineral phase but because it is a mild process in which the incoming ions knock out individual surface atoms it does not result in any detectable gross morphological artefactual damage as produced by other ultra sectioning techniques. Human teeth extracted from young healthy adults 20 to 30 years of age were obtained from the Royal Dental Hospital of Melbourne and fixed in 10% v/v isotonic buffered formal saline for 24 h at room temperature. After washing in water and air drying, the teeth were embedded in Servic polyester resin (obtained from R. F. Service, 272 Brunswick Street, Fitzroy, Melbourne) and cured for 24 h ( $\times 180,000$ ).

We have observed with the electron microscope a micropore structure in human dental enamel using an improved technique in which specimens are prepared by alumina jet dimpling followed by ion beam thinning<sup>4</sup>. This technique enables the preselection of a defined area for preferential thinning, making it suitable for electron transmission.

Longitudinal serial sections (300  $\mu$ m thick) of resin-embedded teeth were prepared by the method of Malcolm<sup>5</sup>. After lapping by hand to approximately 100  $\mu$ m thickness and after polishing both sides, the sections were mounted on glass slides using an alcohol-soluble medium, such as canada balsam. Using a light microscope, areas of enamel were selected in each specimen (a) in the vicinity of the dentino-enamel junction in the cusp region and (b) midway between the surface and the dentino-enamel junction in the central region between cusp tip and the cervix. These areas were then enclosed by cementing a slotted grid or ring to the specimen with a rapid-setting alcohol-insoluble cement. The grid or ring also provides support for the specimen. A dimple (diameter < 1 mm) was made on the area selected by accurate positioning under a jet of fine alumina directed from a fine silicon carbide nozzle. Rate of dimpling was controlled by the nitrogen gas pressure operating the jet; the use of different size jets, combined with varying



specimen-jet distances, enabled determination of the area thinned. A specimen thickness of approximately 30  $\mu\text{m}$  in the dimpled area was found suitable for ion beam thinning; this was measured optically or using a dial gauge. After dimpling, the disk was separated from the glass base by immersion in alcohol and then trimmed with a sharp knife. The disk, mounted in a stainless steel holder, was tilted to approximately  $15^\circ$  to the plasma beam in an ion beam thinning unit. Perforation of the dimpled area (monitored microscopically) was the criteria determining suitability for electron microscopy. The exact identification in the electron microscope (JEM 200 kV) of specific features noted optically was achieved by careful photographic comparison before and after thinning. Electron diffraction was used to determine the precise orientation of enamel crystals in any one field. The transmission electron microscope observations showed that human dental enamel prepared as above had no detectable gross morphological artefactual damage found with other ultra-sectioning techniques.

In longitudinal section, enamel crystals appeared to be long lath or ribbon-like structures extending across the field without interruption, except at prism boundaries. These crystals (approximately 30–90 nm wide) appeared to be of indeterminate length with none of the cross fractures usually observed in electron micrographs of enamel crystals. The most striking feature in all areas of the enamel examined, however, was the presence of electron-lucent channels running between, and parallel to, the crystals (Figs 1 and 2). These channels or pores (1–10 nm wide; average width about 5 nm), like the crystals, appeared to be of indeterminate length in longitudinal sections; they may run for long distances through the enamel rods and

only terminate at a rod boundary or free surface. They were best observed in slightly out of focus micrographs because of enhanced contrast due to Fresnel fringes. But the width measurements were made from in-focus micrographs. In cross section, these channels appeared as irregular shaped holes of similar diameter to that measured for the longitudinally sectioned channels. It was impossible to tell from the electron micrographs whether the channels had a material structure of their own or were merely spaces created by the packing arrangements of the crystals. The electron micrographs gave the impression that these channels were empty spaces, however, they may have contained soft material, for example organic matter, preferentially removed by the ion beam thinning.

An approximate estimate of the relative percentage volume of these pores was made by examining over 200 electron micrographs of numerous fields. The pore area and total area were calculated using a ruled grid on a transparent overlay and then multiplied by average pore diameter and crystal thickness, respectively, obtained from electron micrographs. Determination of the percentage pore volume relative to total enamel volume for a section of enamel one crystal thick gives a range from 1 to 5%, using the widest range of pore diameters.

Although this calculation is approximate, the close agreement with established figures<sup>1</sup> is strong evidence that the pores observed here do represent the spaces in enamel, detected by indirect means.

Experimentation<sup>6-8</sup> has shown that human teeth are permeable to ions and some molecules which can pass from the pulp to the enamel surface or *vice versa*. The presence in the enamel of a system of pores or channels (average diameter 5 nm), which would permit the passage of ions and small molecules, would explain the permeability of this material. It must also have considerable significance for its physico-chemical behaviour, particularly with regard to dissolution, remineralisation and carious destruction. These aspects are being investigated currently by a detailed quantitative analysis of the distribution and size range of these micropores in both normal and pathological enamel from cusp tip to cervix and from surface to dentino-enamel junction.

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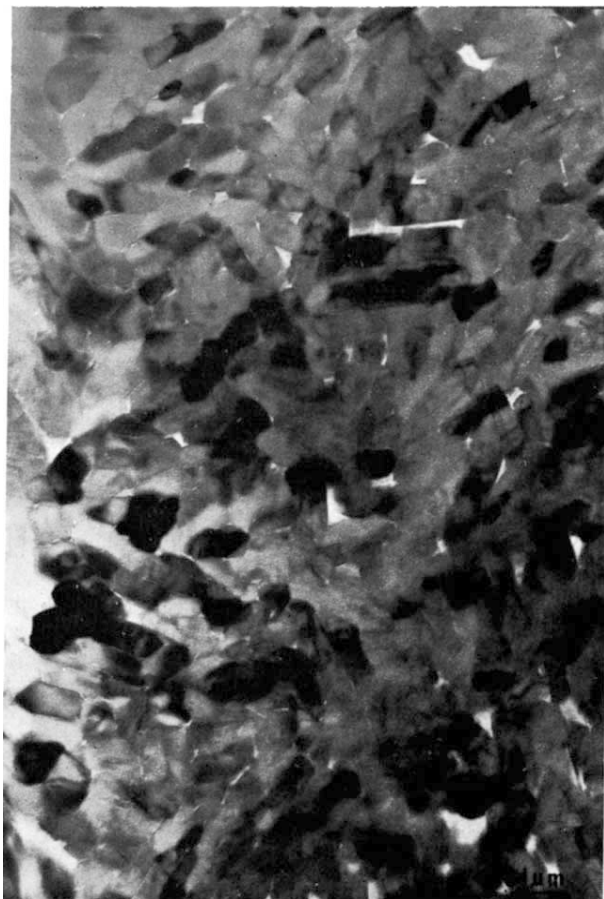


Fig. 2 Transmission electron micrograph of transverse section of ion beam thinned human dental enamel showing flattened hexagonal apatite crystals with irregularly shaped holes separating the individual crystals. ( $\times 80,000$ )

## Oral contraceptive for men

WE have induced reversible infertility, with little effect on libido, in five healthy young men by giving them tablets containing methyltestosterone and ethinyloestradiol. There were no undesirable side effects and we feel that this treatment may offer a practical approach to male oral contraception.

The acceptability of oral contraceptives for women has led many people to investigate the possibility of producing similar products for use by men<sup>1</sup>. The major mechanism of action of synthetic steroid hormones in oral contraceptives is inhibition of ovulation, due to suppression of the mid-cycle surge of gonadotrophins released by the pituitary<sup>2</sup>. Oral contraceptives inhibit spermatogenesis by a similar mechanism, but so far there has been concomitant loss of libido and potency<sup>3</sup>. Clinical results<sup>4-6</sup>, however, have shown that this can be avoided if supplementary androgens are given in combination with an anti-gonadotrophic steroid such as danazol<sup>4</sup>, norgestrienone<sup>5</sup>, or ethynorgestrienone<sup>6</sup>. But clinical testing of such combinations on a large scale cannot begin without extensive toxicological testing with animals<sup>7</sup>. We have therefore reconsidered existing oral hormone products which have been approved for sale in many countries. One group of these products<sup>8</sup> contains combinations of an androgen (usually methyltestosterone: 17 $\alpha$ -methyl-17 $\beta$ -hydroxy-4-androsten-3-one) with an oestrogen (usually ethynylloestradiol: 17 $\alpha$ -ethynyl-1,3,5(10)-estratriene-3,17 $\beta$ -diol). They are sold for treatment of, for example, osteoporosis in men and the symptoms of 'male menopause'.

Initial studies were conducted on two men with osteoporosis who had been receiving products of this type for several months. Both proved to be aspermic, but reported no undesirable side effects, apart from a transitory nausea during the first few days. Concentrations of bilirubin, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, alkaline phosphatase and albumin in these men were within normal limits. With this background, we decided to test an oral androgen-oestrogen product as a contraceptive in healthy male volunteers.

The concentration of testosterone in the plasma of healthy men averages 4-12 ng ml<sup>-1</sup>, with a daily production of 5-10 mg<sup>9</sup>. Methyltestosterone has a longer biological half-life than testosterone, but a 10 mg tablet is necessary to maintain concentrations of 4-15 ng ml<sup>-1</sup> in the plasma for up to 8 h (ref. 10). We therefore used tablets containing 10 mg methyltestosterone plus 20  $\mu$ g ethynylloestradiol. This combination was taken twice daily at 0800h and 1800h with meals. A recent study<sup>11</sup> suggested that this dose of oestrogen is sufficient to suppress pituitary secretion of follicle-stimulating hormone (FSH) in normal adult men. To evaluate possible side effects we compared the active hormone preparation with a placebo in the same patients. As supplies of matching placebo tablets were unavailable, the active tablets were crushed and repacked in gelatin capsules. Identical placebo capsules were packed with the same quantity of lactose. Five healthy male subjects (23-38 yr old) received daily placebo capsules for 3 weeks before taking the hormone product, but were unaware of this. The wives of the subjects all used combined-type oral contraceptives and continued to do so until advised to the contrary.

Blood and semen were collected at the start of the study and after 3, 6, 12, 18 and 24 weeks. Routine biochemical analysis of blood plasma indicated no hepatotoxicity. All semen specimens were normal at the beginning and end of the period when placebo was given, but sperm numbers and motility had decreased significantly by week 12 (that is day 63 of hormone treatment). At this time, four of the subjects were aspermic and remained so for the duration of treatment. The other subject produced an aspermic semen specimen during week 18. Semen specimens were collected at approximately monthly intervals after treatment was stopped. There was no increase in sperm in any of the men for about 15 weeks after the cessation of hormone treatment, but all then began to show a gradual restoration. Normal sperm numbers and motility were found after 35-40 weeks without treatment.

We conclude that the androgen oestrogen combination inhibited spermatogenesis, but that the effect was reversible when treatment stopped. Two subjects reported decreased libido during the initial 8 weeks (which included the placebo

period), but normal libido for the remainder of the study. Another man reported increased libido during the second half of the treatment period, while he and another subject experienced a reduction in libido after treatment was stopped.

Three subjects reported occasional mild nausea, sometimes while they were taking the placebo. No nausea was reported after week 18. There were no changes in skin, hair, breasts or micturition. When the wives of the volunteers stopped taking their oral contraceptives between weeks 18 and 34, no pregnancies occurred, as would have been expected with aspermic men.

Thus we believe that hormone preparations already being marketed for other purposes could be used as oral contraceptives for men. We recommend that these preparations be investigated further to establish the most suitable doses and administration regimen. It seems possible that similar effectiveness could be obtained with discontinuous treatment.

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## LSD as an agonist of dopamine receptors in the striatum

THE mechanism of the hallucinogenic action of (+)-lysergic acid diethylamide (LSD) is still obscure. Its molecular structure (containing both an indole and a phenylethylamine moiety) suggests the possibility of an interaction with brain monoamines. The earliest hypothesis postulated an antagonistic action of LSD at 5-hydroxytryptamine (5-HT) receptors in the brain<sup>1,2</sup>. More recent data rather tend to support the view that the hallucinogen mimics at least some of the effects of endogenous cerebral 5-HT<sup>3-5</sup>. Inhibition of the release of 5-HT is another suggested mechanism of action<sup>6</sup>. An interaction of LSD with dopaminergic transmission has so far not been demonstrated, although the marked attenuation of LSD-induced symptoms by chlorpromazine and other antipsychotic drugs<sup>7-9</sup> may suggest this. Using a modification of the rotational model proposed by Ungerstedt<sup>10</sup> we found that LSD acted as a potent agonist at dopamine receptors in the striatum.

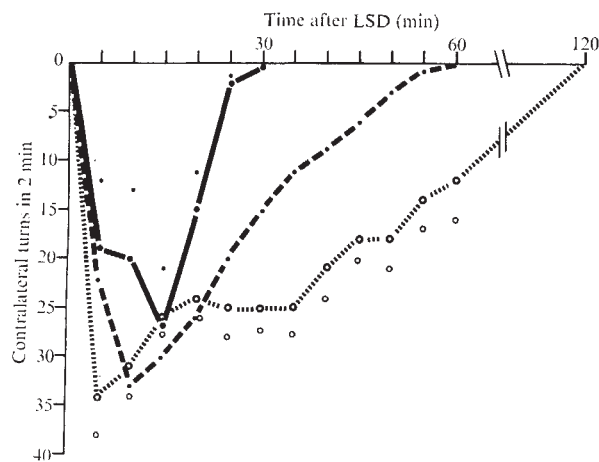
A unilateral lesion of the nigro-striatal dopamine system of rats lowers the dopamine content in the striatum on that side. The resulting imbalance between the dopaminergic activities in the right and left striatum leads to postural and locomotor asymmetry both spontaneously and, even more markedly, in response to certain agents. Thus, drugs which enhance the release of dopamine from nerve terminals in the striatum of the intact side, for example, amphetamine, induce a rotation towards the side with the



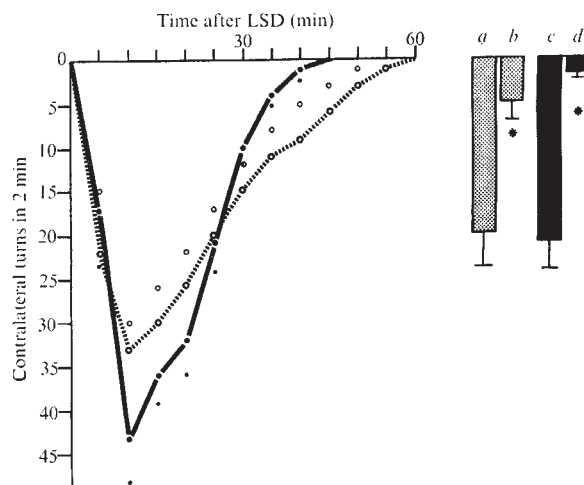
lesion (ipsilateral circling) possibly by removing an inhibitory influence of the striatum upon the motor activity of the side of the body contralateral to the lesion. Conversely, agents like apomorphine, which stimulate dopamine receptors directly, induce rotation towards the intact side (contralateral circling), probably due to exaggerated responsiveness of the denervated striatum to these agents<sup>10</sup>.

Unilateral lesions of the nigro-striatal dopamine system were produced in male SPF rats of the stock Füllinsdorf albino by the injection into the right medial forebrain bundle (MFB) of either 3.5  $\mu\text{g}$  6-hydroxydopamine (6-OH-dopamine) in 4  $\mu\text{l}$  Ringer's solution containing 0.02% ascorbic acid, or 10  $\mu\text{g}$  5,6-dihydroxytryptamine (5,6-HT) in 10  $\mu\text{l}$  of same. Using these two neurotoxic agents we obtained two groups of animals similarly depleted of telencephalic dopamine on the side of the injection but differing in the levels of the other monoamine transmitters: 6-OH-dopamine lowered dopamine and noradrenaline levels (L.P., M.P., and W.H., in preparation), but did not alter the 5-HT content; the injection of 5,6-HT depleted dopamine and 5-HT but did not affect noradrenaline<sup>11,12</sup>. For the measurement of drug-induced behaviour, rats were gently placed in a plexiglas box (35 $\times$ 20 $\times$ 15 cm) immediately following the intraperitoneal (i.p.) injection of the compounds. Handling alone induces in most lesioned animals a 'paradoxical' contralateral rotation<sup>10</sup>, however, which never lasts more than 3 min. The circlings during the first 5 min were therefore not recorded. The intensity of the rotational behaviour was expressed as the total number of full turns counted during 2 min at intervals of 5 min. Beginning on the 10th postoperative day, all rats were tested for their rotational responses to (+)-methamphetamine and apomorphine before LSD was given. A drug-free interval of one week was allowed between tests. As expected, the responses to (+)-methamphetamine (1-5 mg  $\text{kg}^{-1}$  i.p.) and apomorphine (0.05-3 mg  $\text{kg}^{-1}$  i.p.) were ipsilateral and contralateral circling, respectively. Animals treated with 6-OH-dopamine and with 5,6-HT did not differ in their responses. Ten sham-operated rats (local injection of solvent into the MFB) did not rotate in response to apomorphine and amphetamine.

LSD (tartrate) (100  $\mu\text{g}$   $\text{kg}^{-1}$  i.p., but not 50  $\mu\text{g}$   $\text{kg}^{-1}$  i.p.) induced contralateral turning, which began after 5 min and reached a peak after 15 min (Fig. 1). Doses higher than 100  $\mu\text{g}$   $\text{kg}^{-1}$  did not increase further the number of turns per minute but the peak of rotational behaviour was pro-



**Fig. 1** Contralateral rotation (towards the unoperated side) induced by three doses of LSD injected i.p. in rats with lesions of the right medial forebrain bundle produced by the local injection of 5,6-dihydroxytryptamine. The intensity of the rotational behaviour is indicated by the number of turns in 2 min (means of ten animals per dose) s.e.m. Indicated by small points or circles. LSD doses: —, 100  $\mu\text{g}$   $\text{kg}^{-1}$ ; ---, 200  $\mu\text{g}$   $\text{kg}^{-1}$ ; ·····, 1,500  $\mu\text{g}$   $\text{kg}^{-1}$ .



**Fig. 2** Comparison of the rotational response elicited by LSD (200  $\mu\text{g}$   $\text{kg}^{-1}$  i.p.) in rats with lesions of the right medial forebrain bundle produced by 6-hydroxydopamine ( $n=10$ ) and by 5,6-dihydroxytryptamine ( $n=10$ ). The right-hand side shows the effect of haloperidol (1 mg  $\text{kg}^{-1}$ ) injected 10 min after LSD. a and c, Turning activity in the two groups of rats, 25 min after LSD alone; b and d, turning activity under the effect of haloperidol. Means  $\pm$  s.e.m. ( $n=10$ ). The difference between LSD alone and LSD+haloperidol is statistically significant ( $P<0.01$ , Student's  $t$  test, two-tailed). Solid line and bars, 6-hydroxydopamine; dotted line and stippled bars, 5,6-dihydroxytryptamine.

longed in a dose dependent way. The time-course of rotation after LSD (100  $\mu\text{g}$   $\text{kg}^{-1}$ ) paralleled the temporal changes of the levels of LSD in the brain<sup>13</sup>. Repeated injections of LSD (200  $\mu\text{g}$   $\text{kg}^{-1}$  i.p.) on 5 consecutive days, a schedule reported to induce tolerance to the hallucinogen in operant behaviour studies<sup>14</sup>, did not reduce the rotational responses. There was no significant difference between rats lesioned with 6-OH-dopamine and those lesioned with 5,6-HT in the intensity and duration of contralateral circling in response to LSD (200  $\mu\text{g}$   $\text{kg}^{-1}$ ) (Fig. 2). In both groups of animals the injection of haloperidol (1 mg  $\text{kg}^{-1}$  i.p.) 10 min after the administration of LSD (200  $\mu\text{g}$   $\text{kg}^{-1}$ ) significantly reduced the circling behaviour (Fig. 2). Pretreatment 4 h before LSD, with  $\alpha$ -methyl-L-tyrosine ( $\alpha$ -MT) (200 mg  $\text{kg}^{-1}$  i.p.), an inhibitor of the synthesis of catecholamines, did not alter the rotational response in spite of producing marked sedation. (+)-2-Bromo-lysergic acid diethylamide bitartrate (BOL-148), up to 20 mg  $\text{kg}^{-1}$  i.p., was unable to induce circling in either group of lesioned animals. LSD in the doses used had no detectable effect in sham-operated rats.

Thus, in rats with the nigro-striatal dopamine system lesioned unilaterally by injection into the MFB of two different neurotoxic agents (6-OH-dopamine and 5,6-HT), LSD induced circling towards the intact side exactly as does apomorphine. It seems reasonable to conclude that LSD is a potent agonist at central dopamine receptors. Differential depletion of noradrenaline and 5-HT in addition to the reduction of dopamine and the inhibition of catecholamine synthesis by  $\alpha$ -MT had no influence on the effect of LSD; therefore, the mechanism involved in the rotational response to LSD seems to be purely dopaminergic and postsynaptic. Haloperidol, a dopamine receptor antagonist, reduced the effect of LSD as well as that of apomorphine (and of L-dopa). Further support for a dopamine receptor agonistic action of LSD is provided firstly by its slowing down of the turnover of dopamine in the rat brain (M. Da Prada, personal communication), secondly by its ability, though weaker than that of DA, to activate *in vitro* the adenylyl cyclase in a homogenate of rat striatum (W. P. Burkard, personal communication), and finally by the increase of

cyclic adenosine 3',5'-monophosphate in rat cerebrum after its i.p. administration<sup>15</sup>. BOL-148, an analogue of LSD believed to have very weak, if any, hallucinogenic properties<sup>61</sup>, did not induce circling behaviour. In preliminary experiments, methysergide, another non-hallucinogenic lysergic acid derivative, and N,N-dimethyltryptamine, an idolealkylamine hallucinogen, were also inactive as dopamine receptor agonists. On the other hand, such an action has recently been described for some ergot alkaloids and derivatives (ergocornine, 2-bromo- $\alpha$ -ergocryptine, ergometrine)<sup>17,18</sup>.

The activation of striatal dopamine receptors is obviously not the only important central effect of LSD, since its overall behavioural effects differ considerably from those of agents believed to be fairly pure agonists of dopamine receptors. The possible importance of the dopaminergic component of the action of LSD for its hallucinogenic effect has yet to be demonstrated. It will be interesting to see whether LSD also activates dopamine receptors in brain regions other than the striatum, for example, in the limbic subcortical and cortical<sup>18,19</sup> structures, in the hypothalamus, in the medullary chemical trigger zone, and in the retina<sup>22,23</sup>.

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## LSD as an agonist and antagonist at central dopamine receptors

THE mechanisms involved in the psychotomimetic actions of D-lysergic acid diethylamide (D-LSD) and other hallucinogenic agents have not been defined. Neurophysiological and behavioural studies indicate that D-LSD may interact with serotonin and catecholamine receptors in the central nervous system. Thus, this drug seems to stimulate certain central serotonergic pathways<sup>1,2</sup>, while inhibiting the activation of other pathways by serotonin<sup>3,4</sup>.

D-LSD has also been reported to block the action of noradrenaline on central neurones<sup>5</sup> and to produce stereotypy in rats<sup>6</sup>, a condition which is associated with dopaminergic hyperactivity<sup>7</sup>. Moreover, several recent reports have indicated that a number of ergot derivatives are capable of exerting dopamine-like actions on central neurones in the rat<sup>8-10</sup>. Recent investigations from this laboratory suggest that adenylyl cyclase systems may be involved in the central actions of D-LSD (ref. 11 and K.v H., S.R., and D.F., unpublished). Thus, D-LSD is capable of stimulating adenylyl cyclase activity, as well as antagonising the stimulation of this enzyme by serotonin, in cell-free preparations from the rat colliculus. Here we report evidence that D-LSD may act both as an agonist and an antagonist at dopamine receptors in the brain.

The methods employed for the preparation of brain subcellular fractions and measurement of adenylyl cyclase activity have been described in detail elsewhere<sup>12,13</sup>. Brains were obtained from young, adult male rats of an inbred Sprague-Dawley strain. These rats were approximately 6 weeks old and weighed about 225 g. Particulate fractions were prepared from cerebral cortex or corpus striatum.

In relatively low concentration (10  $\mu$ M), D-LSD, completely blocked the stimulation of cerebral adenylyl cyclase activity by a combination of 10  $\mu$ M noradrenaline and 10  $\mu$ M dopamine (Table 1). At these concentrations, the catecholamines produced additive effects on enzyme activity<sup>12</sup>. Another serotonin antagonist, 2-bromo-D-lysergic acid diethylamide (BOL) also inhibited this response to the catecholamines. Comparable results were obtained when these serotonin antagonists at 100  $\mu$ M concentrations were tested against equimolar amounts of either noradrenaline or dopamine. D-LSD also inhibited

**Table 1** Influence of lysergic acid derivatives on activation of adenylyl cyclase by catecholamines in particulate preparations from cerebral cortex of adult rats

| Lysergic acid derivative | Cyclic AMP formed                                     |                                                                              | Increase with catecholamines (nmolmg <sup>-1</sup> protein h <sup>-1</sup> ) (%) |
|--------------------------|-------------------------------------------------------|------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
|                          | Basal (nmolmg <sup>-1</sup> protein h <sup>-1</sup> ) | Increase with catecholamines (nmolmg <sup>-1</sup> protein h <sup>-1</sup> ) |                                                                                  |
| None                     | 8.46 $\pm$ 0.18                                       | 6.39 $\pm$ 0.22                                                              | 76                                                                               |
| D-LSD, 10 $\mu$ M        | 9.46 $\pm$ 0.26                                       | 0.31 $\pm$ 0.29*                                                             | 3                                                                                |
| L-LSD, 80 $\mu$ M        | 8.02 $\pm$ 0.15                                       | 5.63 $\pm$ 0.49                                                              | 70                                                                               |
| BOL, 10 $\mu$ M          | 7.60 $\pm$ 0.28                                       | 0.91 $\pm$ 0.41*                                                             | 12                                                                               |

The particulate preparations were crude mitochondrial fractions obtained by centrifuging the 1,000g supernatant fluids from cerebral homogenates at 10,000g for 20 min. Adenylyl cyclase activity was determined from the conversion of <sup>14</sup>C-ATP to cyclic AMP by a procedure<sup>12</sup> which was based on the method of Krishna *et al.*<sup>14</sup>. The incubation medium (0.1 ml) contained the following components in the final concentrations indicated: 3.6 mM (0.5  $\mu$ Ci) <sup>14</sup>C-ATP, 5.0 mM MgCl<sub>2</sub>, 1.0 mM cyclic AMP, 40 mM Tris-HCl (pH 7.3 at 37°C), 10 mM phosphoenolpyruvate, 4  $\mu$ g pyruvate kinase, 15 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (added with pyruvate kinase), 20 mM caffeine, 0.1 mg bovine serum albumin, 0.2 mM EGTA, 5  $\mu$ g phosphatidylserine and 50-100  $\mu$ g brain protein. Protein was measured by the method of Lowry *et al.*<sup>15</sup>. L-Noradrenaline (10  $\mu$ M) and dopamine (10  $\mu$ M) were added together as the hydrochlorides, D-LSD and L-LSD as the free amines and BOL as the bitartrate. Incubation was conducted in air for 5 min. Values for cyclic AMP formed are averages  $\pm$  s.e. for triplicate samples in a representative experiment. Where indicated, basal values shown were obtained in the presence of the lysergic acid derivative.

\* $P < 0.001$  for the difference between this value and the value obtained with only the catecholamines added.



**Table 2** Influence of blocking agents on activation of adenylyl cyclase by dopamine and D-LSD in particulate preparations from corpus striatum of adult rats

| Agent                   | Basal<br>(nmol mg <sup>-1</sup><br>protein h <sup>-1</sup> ) | Cyclic AMP formed                                                             |     | Increase with D-LSD                                 |     |
|-------------------------|--------------------------------------------------------------|-------------------------------------------------------------------------------|-----|-----------------------------------------------------|-----|
|                         |                                                              | Increase with dopamine<br>(nmol mg <sup>-1</sup><br>protein h <sup>-1</sup> ) | (%) | (nmol mg <sup>-1</sup><br>protein h <sup>-1</sup> ) | (%) |
| Experiment 1            |                                                              |                                                                               |     |                                                     |     |
| None                    | 18.43 ± 0.67                                                 | 11.46 ± 0.76                                                                  | 62  | 5.65 ± 0.73                                         | 31  |
| D-LSD (10 µM)           | 24.08 ± 0.27                                                 | 1.12 ± 0.42*                                                                  | 5   |                                                     |     |
| Chlorpromazine (10 µM)  | 17.34 ± 0.31                                                 | 0.49 ± 0.76†                                                                  | 3   | 1.40 ± 0.42†                                        | 8   |
| Haloperidol (10 µM)     | 16.93 ± 0.32                                                 | 0.73 ± 0.44*                                                                  | 4   | 1.40 ± 0.33†                                        | 8   |
| Experiment 2            |                                                              |                                                                               |     |                                                     |     |
| None                    | 15.57 ± 0.42                                                 | 9.54 ± 0.69                                                                   | 61  | 4.70 ± 0.45                                         | 30  |
| MLD (100 µM)            | 15.03 ± 0.26                                                 | 0.20 ± 0.43*                                                                  | 1   | -0.10 ± 0.45†                                       | -1  |
| BOL (100 µM)            | 15.13 ± 0.48                                                 | -1.70 ± 0.52*                                                                 | -11 | -1.14 ± 0.51†                                       | -8  |
| Propranolol (100 µM)    | 16.03 ± 0.29                                                 | 8.13 ± 0.51                                                                   | 51  | 3.90 ± 0.57                                         | 24  |
| Cyproheptadine (100 µM) | 13.56 ± 0.12                                                 | 0.58 ± 0.37*                                                                  | 4   | 1.31 ± 0.31†                                        | 10  |

The particulate preparations were obtained by centrifuging the homogenates at 10,000g for 20 min. See Table 1 for other explanations.

\* $P < 0.001$  for the difference between this value and the value obtained with only 10 µM dopamine or 10 µM D-LSD added.

† $P < 0.01$ .

the adenylyl cyclase response to noradrenaline or dopamine in particulate preparations from the rat hippocampus, which resembles cerebral cortex in the responses of adenylyl cyclase to the catecholamines<sup>13</sup>. Palmer and Burks<sup>16</sup> earlier reported that D-LSD and BOL block the noradrenaline-induced increase in cyclic AMP levels in slices of rat brain.

By itself, D-LSD tended to increase adenylyl cyclase activity in the cerebral and hippocampal preparations. This effect was quite small however, (about 15%) and was not always significant. A survey of various brain regions of the rat revealed that cell-free fractions from the corpus striatum contained adenylyl cyclase which was reproducibly activated by low concentrations of D-LSD. Significant stimulation could be obtained with concentrations of D-LSD as low as 0.1 µM. Adenylyl cyclase activity in comparable fractions from other regions of rat brain (cerebellum, hypothalamus and brainstem) was completely refractory to the stimulatory action of this drug.

Adenylyl cyclase activity in particulate preparations from striatal tissue of the adult rat was increased about 30% by 10 µM D-LSD (Table 2). This was about one-half the activation produced by an equimolar concentration of dopamine. The sensitivity of adenylyl cyclase to dopamine in cell-free fractions from rat corpus striatum has been demonstrated earlier<sup>13,17,18</sup>. This region of rat brain is unusually rich in dopaminergic neurones<sup>19</sup>. The response to 10 µM dopamine in our investigations was completely blocked by the addition of 10 µM D-LSD. In addition, the responses of adenylyl cyclase to either 10 µM dopamine or 10 µM D-LSD were almost completely blocked by equimolar concentrations of the antipsychotic dopamine-blocking agents, haloperidol and chlorpromazine. The serotonin antagonists, BOL, 1-methyl-D-lysergic acid diethylamide (MLD) and cyproheptadine were also capable, however, of inhibiting these responses (Table 2, experiment 2). The β-adrenergic blocking agent, propranolol, which markedly inhibits the response of adenylyl cyclase to noradrenaline in rat brain cell-free preparations<sup>12</sup>, did not block the stimulation of striatal adenylyl cyclase by dopamine or D-LSD. None of these blocking agents by itself, with the exception of D-LSD, increased adenylyl cyclase activity in the striatal preparations.

Our findings, combined with earlier observations from this laboratory<sup>11</sup>, indicate that D-LSD is capable of blocking the interaction of noradrenaline, dopamine and serotonin with their respective receptors in various regions of rat brain. This drug also seems to activate central serotonin and dopamine receptors for adenylyl cyclase. Interaction of D-LSD with dopamine receptors was blocked by dopamine-blocking agents (for example, haloperidol) or serotonin antagonists (for example, cyproheptadine), but not by the noradrenaline antagonist, propranolol. These investigations suggest that the hallucinogenic actions of D-LSD may be due to complex agonist and antagonist actions of this drug at central receptors for the several neurotransmitter monoamines.

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## The activity of membrane bound enzymes in muscular dystrophic chicks

TISSUES from patients with Duchenne muscular dystrophy<sup>1</sup> (DMD) as well as animals with similar inherited<sup>2</sup> or experimentally produced<sup>3</sup> disorders have been shown to undergo

**Table 1** Adenyl cyclase in dystrophic chick muscle sarcolemma (pmol cyclic AMP per mg protein per 5 min)

| Activity | 16 day embryos |            |       | 8 days   |            |       | 6 weeks  |            |       |
|----------|----------------|------------|-------|----------|------------|-------|----------|------------|-------|
|          | Normal         | Dystrophic | P     | Normal   | Dystrophic | P     | Normal   | Dystrophic | P     |
| Basal    | 29.8±2.3       | 5.7±1.3    | <0.01 | 28.0±2.7 | 29.5±0.1   | NS    | 20.1±0.5 | 58.7±7.8   | <0.01 |
| GTP      | 37.8±0.4       | 17.6±2.5   | <0.01 | 32.3±0.9 | 38.9±3.6   | <0.05 | 28.8±0.7 | 102.8±13.6 | <0.01 |

Muscle sarcolemma was prepared from the pectoralis of pooled muscular dystrophic chicks and age-matched white leghorn (No. 26 from Arbor Acres) control chicks by the method of Severson *et al.*<sup>15</sup>. Adenyl cyclase was assayed by measuring the conversion of  $\alpha$ -<sup>32</sup>P-ATP into 3',5'-cyclic-AMP<sup>16</sup>. Membrane protein (100–150  $\mu$ g per assay tube) were incubated for 5 min at 37°C at pH 8.3. Protein was determined by the method of Lowry *et al.*<sup>17</sup> using bovine serum albumin as standard. Each estimate represents the mean  $\pm$  s.d. of three determinations. Statistical significance of the differences between means was determined using Student's *t* test.

significant changes in the amount and composition of their membrane lipids. Most pronounced are the increases in cholesterol and sphingomyelin and the decrease in phosphatidyl choline levels. Functional anomalies observed in this disease, such as the impairment in sarcoplasmic reticulum calcium uptake<sup>4</sup> or the changes in the ATPase activity of the sarcolemma<sup>5</sup>, can be related to the lipid disorder.

Both the anomaly in lipid composition and related functional impairments have been observed in tissues other than muscle. For example, erythrocytes from patients with DMD were shown to differ from controls in their lipid composition<sup>1</sup>, in their surface properties as characterised by scanning electron microscope<sup>6</sup> and in membrane ATPase activity<sup>7</sup>. Similarly, in mice with muscular dystrophy the erythrocyte acetylcholine esterase activity was found to be half that of normal controls<sup>8</sup>. Such observations led to the hypothesis that the genetic basis of Duchenne muscular dystrophy is a defect in lipid metabolism<sup>1,2,9</sup> which may involve the plasma membrane<sup>4,10,11</sup>. Lipids are instrumental not only in the control of membrane selectivity and diffusion across the membrane<sup>12</sup> but also in the determination of the activity of membrane bound enzymes and their modulation by hormones and other agents<sup>13,14</sup>. One would therefore expect that if muscular dystrophy is due to a lipid related plasma membrane disorder the activity of membrane associated enzymes and their susceptibility to stimulation should be affected. Involvement of multiple organs would support the hypothesis that the membrane defect is linked to the genetic cause of the disease.

In this study we have investigated adenyl cyclase and ATPase activity associated with the plasma membrane of muscle, liver and erythrocytes from dystrophic chicks and the appropriate controls, under conditions of enzyme stimulation and interference with membrane cholesterol. The adenyl cyclase activity of the muscle sarcolemma from dystrophic chicks differed significantly from that of age-matched normal chicks in several

respects (Table 1). Whereas in the normal chick the specific activity of the enzyme did not vary considerably with age, in the dystrophic it increased progressively to reach, at six weeks, twice the control level. The enhanced GTP ( $10^{-5}$  M) stimulation of the enzyme in the dystrophic membrane (Table 1) suggests that the differences are due to the expression of enzyme activity rather than to the amount of enzyme present.

Enzyme activity is determined by the microenvironment which controls the conformation of the enzyme, its coupling to the stimulus-receptor site and the substrate accessibility. It has been shown recently that this microenvironment can be affected by amphotericin B, which interacts with cholesterol<sup>14</sup>. Addition of amphotericin B to the sarcolemma depressed the basal enzyme activity of the normal muscle more than that of the dystrophic one. Also, the ratio of the GTP-stimulated to the basal enzyme activity increased after amphotericin B treatment in the normal membrane but not in the dystrophic one (Table 2).

Significant differences were also observed in the adenyl cyclase of liver plasma membrane. As in the muscle of 6-week-old chicks, the basal enzyme activity was elevated in the dystrophic chicks. The percentage stimulation by  $10^{-5}$  M glucagon increased after amphotericin B treatment in dystrophic liver but decreased substantially in the normal liver membrane (Table 2). This reiterated the role of the lipid environment in the mediation of the hormone stimulation. Differences between liver and muscle membrane may be due either to tissue specificity or to the mode of action of the different stimulating agents.

In red blood cell membrane, the basal adenyl cyclase activity was lower in dystrophic chicks and showed substantially less stimulation by adrenaline. Digitonin treatment, which extracts membrane cholesterol, reduced the enzyme activity in the normal membrane and totally abolished it in the dystrophic membrane (Table 2).

**Table 2** The effect of amphotericin B or digitonin on the plasma membrane adenyl cyclase of normal and dystrophic chicks (pmol cyclic AMP per mg protein per 5 min)

| Activity     | Control membrane |            |                  | Treated membrane |            |                  |
|--------------|------------------|------------|------------------|------------------|------------|------------------|
|              | Basal            | Stimulated | Stimulated/Basal | Basal            | Stimulated | Stimulated/Basal |
| Tissue       |                  |            |                  |                  |            |                  |
| Muscle       |                  |            |                  |                  |            |                  |
| Normal       | 28.0±2.7         | 32.3±0.9   | 1.15±0.09        | 17.7±0.6         | 24.0±0.3   | 1.34±0.03        |
| Dystrophic   | 29.5±0.1         | 38.9±3.6   | 1.32±0.07        | 24.5±2.5         | 30.0±0.5   | 1.22±0.08        |
|              | NS               | P<0.05     |                  | P<0.01           | P<0.01     |                  |
| Liver        |                  |            |                  |                  |            |                  |
| Normal       | 111.9±2.2        | 200.7±5.6  | 1.79±0.02        | 100.3±1.3        | 128.6±5.4  | 1.28±0.03        |
| Dystrophic   | 136.9±9.6        | 215.9±7.8  | 1.58±0.05        | 98.1±3.0         | 167.8±2.6  | 1.71±0.02        |
|              | P<0.02           | P<0.05     |                  | NS               | P<0.01     |                  |
| Erythrocytes |                  |            |                  |                  |            |                  |
| Normal       | 3.50±0.17        | 6.13±0.65  | 1.75±0.07        | 0.80±0.06        | 0.51±0.05  | 0.90±0.19        |
| Dystrophic   | 1.49±0.02        | 1.87±0.30  | 1.20±0.12        | 0                | 0          | —                |
|              | P<0.01           | P<0.01     |                  |                  |            |                  |

Muscle sarcolemma was prepared by the method of Severson *et al.*<sup>15</sup>. Liver plasma membrane was prepared following the procedure of Neville<sup>18</sup> up to step 11 (partially purified membranes). Chick erythrocyte membranes were prepared by using a modification of the procedure of Oye and Sutherland<sup>19</sup>. Adenyl cyclase activity in muscle was assayed as described in Table 1. Liver plasma membrane protein (50–100  $\mu$ g per assay tube) was incubated for 5 min at 37°C at pH 7.6. Erythrocyte membrane protein (300–350  $\mu$ g per assay tube) was used under the same conditions. Muscle adenyl cyclase was stimulated with  $10^{-5}$  M GTP, liver with  $10^{-5}$  M glucagon and erythrocyte membrane with  $10^{-5}$  M adrenaline. Muscle and liver plasma membranes were treated with amphotericin B (Squibb intravenous preparation) to yield a concentration of 0.1 mM. Erythrocyte membrane was treated with 1 mg ml<sup>-1</sup> digitonin as described by Pohl *et al.*<sup>13</sup>. Results represent the mean  $\pm$  s.d.m. of three determinations. Statistical significance of the differences between means was determined using Student's *t* test. s.d. of the ratios, R, was computed according to  $\sigma_R = [(\sigma^2_{r1}/r^2_1) + (\sigma^2_{r2}/r^2_2)]^{1/2}$ , where  $R = r_1/r_2$  and where values of  $\sigma$  are the respective s.d.



**Table 3** The effect of amphotericin B on the muscle and liver plasma per membrane ATPase of normal and dystrophic chicks ( $\mu\text{mol Pi}$  per mg protein per 20 min)

| Condition  | Control membrane                        |                 |                    | Amphotericin B treated                  |                 |                    |
|------------|-----------------------------------------|-----------------|--------------------|-----------------------------------------|-----------------|--------------------|
|            | $\text{Na}^+-\text{K}^+-\text{Mg}^{2+}$ | + Ouabain       | + $\text{Ca}^{2+}$ | $\text{Na}^+-\text{K}^+-\text{Mg}^{2+}$ | + Ouabain       | + $\text{Ca}^{2+}$ |
| Tissue     |                                         |                 |                    |                                         |                 |                    |
| Muscle     |                                         |                 |                    |                                         |                 |                    |
| Normal     | $1.86 \pm 0.01$                         | $1.83 \pm 0.01$ | $2.43 \pm 0.04$    | $2.07 \pm 0.04$                         | $2.26 \pm 0.02$ | $2.40 \pm 0.02$    |
| Dystrophic | $1.21 \pm 0.05$                         | $0.97 \pm 0.01$ | $1.34 \pm 0.01$    | $1.21 \pm 0.01$                         | $1.23 \pm 0.03$ | $1.52 \pm 0.02$    |
|            | $P < 0.01$                              | $P < 0.01$      | $P < 0.01$         | $P < 0.01$                              | $P < 0.01$      | $P < 0.01$         |
| Liver      |                                         |                 |                    |                                         |                 |                    |
| Normal     | $10.7 \pm 0.19$                         | $8.9 \pm 0.16$  | $9.1 \pm 0.20$     | $10.1 \pm 0.19$                         | $6.4 \pm 0.08$  | $6.2 \pm 0.12$     |
| Dystrophic | $6.4 \pm 0.23$                          | $6.7 \pm 0.07$  | $6.6 \pm 0.55$     | $6.4 \pm 0.10$                          | $7.3 \pm 0.16$  | $6.2 \pm 0.16$     |
|            | $P < 0.01$                              | $P < 0.01$      | $P < 0.01$         | $P < 0.01$                              | $P < 0.01$      | NS                 |

Plasma membrane of muscle and liver and their treatment with amphotericin B, were as described in Table 2. ATPase activity was assayed by incubating 100  $\mu\text{g}$  protein per assay tube for 20 min at  $37^\circ\text{C}$  in 40 mM imidazole, 10 mM Tris-HCl, 2 mM Mg (pH 7.4) in the presence of 100 mM NaCl and 10 mM KCl to which either 0.1 mM ouabain or 0.2 mM calcium were added. The ATP concentration was 1 mM. Inorganic phosphate was determined colorimetrically using the ammonium molybdate reaction<sup>20</sup>. The results represent the mean  $\pm$  s.d. of three determinations. Statistical significance of the differences between means was determined using Student's *t* test.

The  $\text{Na}^+-\text{K}^+-\text{Mg}^{2+}$ -ATPase activity of the dystrophic chick muscle sarcolemma was reduced relative to control and was less stimulated by calcium, as previously observed<sup>5</sup>. Of particular interest were the substantial differences observed in the ATPase activity of the liver plasma membranes. As in muscle, the basal level was lower in dystrophic chicks. Ouabain moderately inhibited the ATPase activity of the normal membrane and slightly enhanced that of the dystrophic one. This difference became more prominent after amphotericin B treatment, when ouabain stimulation was clearly observed in the dystrophic membrane. ATPase stimulation by ouabain has also been observed in erythrocyte membrane from DMD patients<sup>7</sup>. Calcium ( $10^{-4}\text{M}$ ) had a similar effect to that of ouabain.

Although it is difficult at present to fit the various differences between the membrane of normal and diseased chicks into a single comprehensive molecular model, the findings support the hypothesis of a generalised membrane defect as an underlying genetic disorder in muscular dystrophy, making it a possible eukaryote membrane mutant. The effects of amphotericin B and digitonin are consistent with the possibility that the basis of this defect is a disorder in lipid metabolism and/or lipid assembly into the plasma membrane.

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## Voltage clamp studies of glutamate synapse

THERE is considerable evidence that L-glutamate is the excitatory transmitter both at the insect and crustacean neuromuscular junction<sup>1,2</sup>, and also in the vertebrate central nervous system<sup>3</sup>. Here we report the use of the voltage clamp technique to compare the reversal potentials of the conductance increase produced by the excitatory transmitter and the application of L-glutamate at the insect neuromuscular junction. We also investigated the ionic basis of the L-glutamate conductance increase using voltage clamping. These experiments could be carried out accurately because changes in resistance of the electrically excitable membrane caused by changes in membrane potential or by different ionic media do not affect the amplitude of the excitatory junctional currents or glutamate currents.

We studied the extensor tibiae muscle of the metathoracic leg of the locust *Schistocerca gregaria*. The muscle fibres were clamped using a two microelectrode method, with a potential recording electrode connected to a current passing electrode through a high input impedance amplifier and a feedback amplifier. The smallest muscle fibres which have a length of 1.5 mm and a diameter of 100  $\mu\text{m}$  could be clamped with only 2% error using this technique.

In normal saline (NaCl 170.0, KCl 10.0,  $\text{CaCl}_2$  2.0, HEPES buffer 10.0 mM  $\text{l}^{-1}$ ; pH 6.8), resting potentials of 55-60 mV were recorded. The neurally-evoked responses were reduced in amplitude by the addition of 40 mM Mg to the saline. Stimulation of the excitatory axon to the muscle with a suction electrode produced a transient depolarisation in the unclamped fibre, the excitatory junctional potential (e.j.p.). In the clamped fibre, nerve stimulation produced an inward current, the excitatory junctional current (e.j.c.). An e.j.p. and e.j.c. are shown in Fig. 1. The e.j.c.s had a maximum amplitude of  $1 \times 10^{-6}\text{A}$  and an average rise time of 3.9 ms, a much shorter time course than the e.j.p. L-glutamate was applied to the muscle iontophoretically, using high resistance electrodes which obviated the use of braking currents. Iontophoresis of L-glutamate on to the junctional region of the muscle produced a transient inward current of maximum amplitude  $1 \times 10^{-7}\text{A}$ . The rise time of the glutamate currents varied

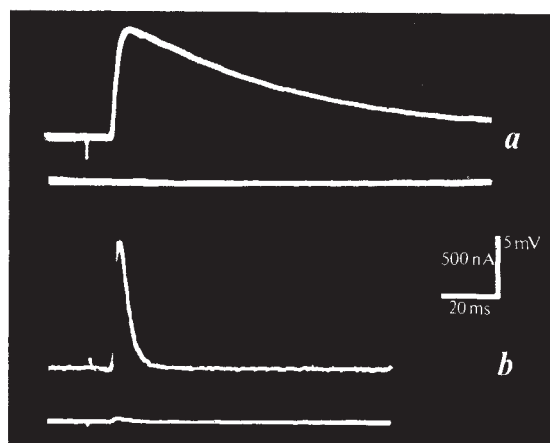
between 20–100 ms, depending on the distance of the iontophoretic electrode from the junction.

Reversal potentials were determined by clamping the e.j.c. and glutamate current at different membrane potentials. The currents were reduced in amplitude as the membrane was depolarised. Actual reversal of the sign of the currents was difficult to achieve because of muscle contraction. Consequently, most reversal potentials were obtained by extrapolation. A linear relationship was obtained for the e.p.c.s and the glutamate currents between 0 mV and 60 mV. The reversal potential of the e.j.c. was  $+3.4 \pm 5.2$  mV (mean  $\pm$  s.d., eight experiments in eight muscles), and the reversal potential of the glutamate current was  $+2.7 \pm 2.4$  mV (mean  $\pm$  s.d., eight experiments in eight muscles). Sometimes a slow outward current was observed following the inward glutamate current when the membrane was depolarised. This is probably due to stimulation of extrajunctional glutamate receptors causing an increase in permeability to Cl (ref. 4).

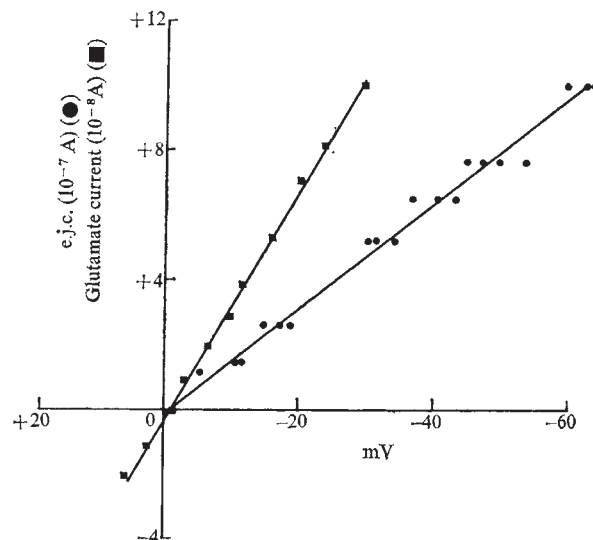
Takeuchi and Onodera<sup>5</sup> have recently obtained a close agreement between the reversal potentials of the e.p.c. and the glutamate current at the crustacean neuromuscular junction. In insects, reversal values of between  $-10$  mV and  $-25$  mV have been obtained for the glutamate potential and the miniature excitatory postsynaptic potentials at the locust neuromuscular junction<sup>6</sup>. Moreover, reversal values of  $+6$  mV for the excitatory junctional potential and  $+7$  mV for the glutamate potential have also been obtained by reversal of sign at the locust neuromuscular junction (S. G. Cull-Candy and P.N.R.U., unpublished).

Perfusion with Na-free (choline Cl substituted) saline caused a 90–95% reduction in the amplitude of the junctional glutamate current. The reversal potential in the Na-free saline varied between  $-10$  and  $-15$  mV. Changes in the K concentration of the saline between 0 mM and 20 mM did not alter the reversal potential of the glutamate current. A reduction in the external K from 10 to 0 mM doubled the amplitude of the current, however, the opposite of that expected if K contributed to the current. The reversal potential of the junctional current was also unchanged in Cl free saline. Increasing Ca from 2 to 50 mM in normal saline reduced the amplitude of the glutamate current by about 90%, but the small glutamate current which can be recorded in Na-free saline increased in amplitude by up to six times when Ca was increased from 2 to 50 mM.

As the reversal potentials for the e.p.c. and the glutamate current were close to zero, it seemed likely that they were the resultants of a simultaneously occurring inward Na and outward K or Cl currents, as the equilibrium potentials for Na,



**Fig. 1** *a*, e.j.p. Recorded intracellularly from an extensor tibiae muscle fibre of the locust. *b*, Upper record, (e.j.c.) recorded from the same muscle fibre with the membrane potential held at the resting level using voltage clamping; lower record, clamped membrane potential. Only a small deviation of 0.1–0.2 mV is seen after nerve stimulation.



**Fig. 2** Relationship between the membrane potential and the amplitude of the e.j.c. (●) and glutamate current (■).

K and Cl in locust muscle have been theoretically calculated as  $+43$  mV,  $-61$  and  $-50$  mV, respectively<sup>7</sup>. The e.p.c.s and acetylcholine currents at the vertebrate neuromuscular junction have reversal potentials between  $-10$  mV and  $-20$  mV, and are generated by simultaneous inward Na currents and outward K currents<sup>8,9</sup>.

The results of our study, however, suggest that the glutamate current consists only of an inward current carried by Na, with Ca perhaps making a small contribution. K and Cl do not seem to carry any outward current, as alteration of external K and Cl does not change the reversal potential. The absence of any outward current is difficult to explain if the theoretically calculated  $E_{Na}$  for locust muscle is correct. Perhaps the intracellular or extracellular concentrations of Na at the synaptic region are different from the non-synaptic regions, so that  $E_{Na}$  at the synaptic region is close to 0 mV. It is also surprising that a large change in the concentration of external Na only causes a small shift in the reversal potential. This may be due to Ca, which has a very positive equilibrium potential, carrying a greater proportion of the current as external Na is lowered.

Another explanation of the results is that both Na and K do participate in the glutamate current, but the sodium and potassium conductance ratio,  $\Delta g_{Na}/\Delta g_K$  varies as the ionic media is altered so that the reversal potential remains at or near zero. Thus as external Na is reduced,  $\Delta g_{Na}/\Delta g_K$  and  $\Delta g_{K_{Ca}}$  increases, while as external K is reduced,  $\Delta g_{Na}/\Delta g_K$  decreases. At the vertebrate muscle end-plate,  $\Delta g_{Na}/\Delta g_K$  stayed constant with most changes in the ionic media, but did decrease markedly when the external K was increased above 10 mM (ref. 9).

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## Synapse formation between clonal muscle cells and rat spinal cord explants

ALTHOUGH functional neuromuscular junctions have been reported between cultured muscle and spinal cord explants<sup>1–3</sup> or dissociated spinal cord cells<sup>4</sup>, further studies have been hampered because primary cultures represent a mixture of cell types of unknown function. Harris *et al.*<sup>5</sup> tackled this problem by coculturing a neuronal cell line (C1300) from a mouse sympathetic ganglion cell<sup>6,7</sup> with a rat skeletal muscle cell line (L6)<sup>8</sup>. They found regions of high acetylcholine (ACh) sensitivity at points of contact between nerve and muscle, indicating interaction between nerve and muscle<sup>6,9</sup>. So far no functional synapses have been observed in this system. We now report that the L6 muscle cell line can form functional synapses with a competent nerve.

The L6 cell line culture and intracellular recording techniques for monitoring the membrane potential were as reported previously<sup>5,9,10</sup>. Electrophysiology was done at room temperature (20°–24° C) in the following saline (NaCl, 142.6 mM; KCl, 5.6 mM; CaCl<sub>2</sub>, 10 mM; HEPES (N-2 hydroxyethyl-piperazine-N'-2-ethansulphonic acid)-NaOH, 5 mM, pH 7.4). In some experiments electrode noise was minimised to 100  $\mu$ V peak-to-peak by using a rather low resistance pipette (50 M $\Omega$ ) and a low pass filter (time constant 0.5 or 1 ms).



Fig. 1 A microscope picture showing a few nerves making contact with an L6 myotube. The bar at the right corresponds to 50  $\mu$ m.

Spinal cords were dissected from 10–20-d-old rat foetuses. Connective tissue was removed and the cord was cut into pieces, about 0.1 mm<sup>3</sup>, with a pair of needles. Several pieces were placed on a monolayer of myotubes with 0.1–0.5 ml of culture medium and incubated for 1–3 h to allow the explant to stick to the underlying cells. Then modified Eagle's medium<sup>11</sup> supplemented with 10% foetal calf serum was added. During the next few days processes grew out of the explant and several rapidly dividing cells began to be seen, probably fibroblasts and myoblasts. To reduce their proliferation *d*-arabinofuranosylcytosine ( $5 \times 10^{-5}$  M) was added after 3–5 d (ref. 4). After 5–7 d the cultures were returned to the original medium.

At various stages of coincubation the myotubes were penetrated with microelectrodes to record miniature end-plate potentials (m.e.p.p.s.). Figure 1 shows a typical nerve muscle pair in which the muscle is contacted by a few nerves. The m.e.p.p.s. were first observed after 7 d, usually a few per minute. After 2 weeks many myotubes produced m.e.p.p.s. 6–60 per min (Fig. 2 shows sample traces).

The cholinergic nature of these m.e.p.p.s. was demonstrated by applying *d*-tubocurarine ( $0.3$ – $10 \times 10^{-7}$  M). The half blocking dose was about  $4 \times 10^{-7}$  M, larger than that found for rat neuromuscular junction ( $3 \times 10^{-8}$  M) but similar to that found

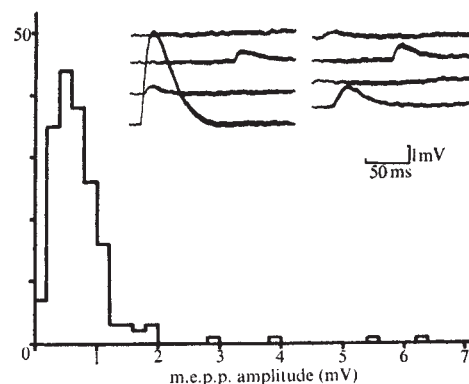


Fig. 2 Amplitude histogram of m.e.p.p.s. Sample recordings of m.e.p.p.s.

for denervated rat muscle<sup>12</sup> and for the potential changes evoked by iontophoretic application of ACh in the L6 cell line<sup>13</sup>.  $\alpha$ -Neurotoxin isolated from an Indian cobra, *Naja naja*, also blocked m.e.p.p.s. at a concentration of  $4 \times 10^{-7}$  g ml<sup>-1</sup>; this is similar to the concentration required to block the response of L6 myotubes to iontophoretically applied ACh<sup>14</sup>.

To determine whether these synapses contain functional acetylcholinesterase the effect of an acetylcholinesterase inhibitor was examined. Edrophonium chloride (Tensilon, Hoffman La Roche<sup>15,16</sup>) at 3.8 to 100  $\mu$ M did not increase the amplitude, the time-to-peak or the half-decline time of m.e.p.p.s. recorded in cultures after 15–31 d of coincubation. This result agrees with observations on similar systems<sup>4</sup>. Koenig<sup>17</sup> has reported histochemical evidence, however, for the presence of acetylcholinesterase in this system.

The m.e.p.p. amplitude and time course varied over a large range. For example, in the fibre shown in Fig. 2, the amplitudes varied from 0.2 mV to 6.3 mV, the time-to-peak from 6 to 26 ms and the half-decline time from 8.5 to 31 ms. The amplitude histogram which was obtained in the presence of tetrodotoxin ( $6 \times 10^{-6}$  M) to block end-plate potentials (e.p.p.s.), is skewed (in Fig. 2 the coefficient of skewness is 4.6). A similar skewed distribution was found in neuromuscular junctions formed *in vitro*<sup>4</sup> and regenerating neuromuscular junctions<sup>18,19</sup>.

The mean m.e.p.p. amplitude was also variable from cell to cell. There was a roughly linear correlation between the steady input resistance of the L6 myotube and the mean amplitude of the m.e.p.p.s. recorded in that myotube (in

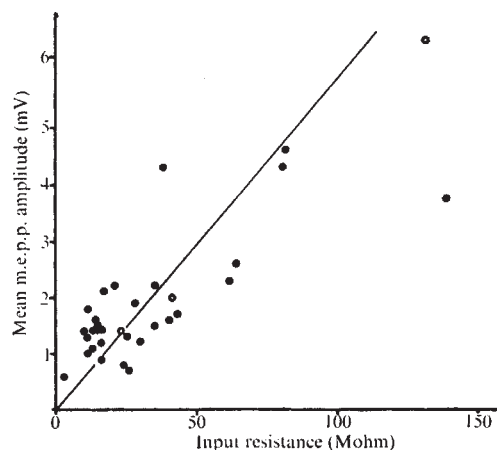


Fig. 3 Correlation between the steady state input resistance and the mean m.e.p.p. amplitude. The m.e.p.p. amplitude is corrected for the resting potential by multiplying by a factor  $64/(V-5)$  (where  $V$  is the absolute value of the resting potential in mV). The standard resting potential is taken as  $-69$  mV and the equilibrium potential for the m.e.p.p. was assumed to be  $-5$  mV (ref. 13). Open circles were obtained in preparations treated with La (about 0.1 mM). The straight line is drawn by eye.

Fig. 3 the correlation coefficient is 0.83). The slope of the regression line for L6 myotubes is 0.05 mV for 1 M $\Omega$  of input resistance (Fig. 3). This is 3.7% of that found for the frog (1.33 mV per M $\Omega$ )<sup>15</sup>. The maximum current during the m.e.p.p.s could be estimated from the maximum rate of rise of the m.e.p.p.s. and the input capacitance of the postsynaptic cells ignoring the current flowing through the resistive component<sup>20</sup>. The maximum rate of rise of the average sized m.e.p.p. in the cell shown in Fig. 2 was 0.19 V s<sup>-1</sup> while the input capacitance obtained by analysing the time course of the membrane potential after applying a small amount of hyperpolarising current, was 1.6 nF. The maximum current was then  $0.3 \times 10^{-9}$  A. Assuming an equilibrium potential for the m.e.p.p.s. of -5 mV, the conductance change during average sized m.e.p.p. was calculated to be  $7.6 \times 10^{-9}$  mho ( $3.5$  to  $10.0 \times 10^{-9}$  mho in six cells). This is 5% of the conductance increase measured in frog neuromuscular junction ( $1.5 \times 10^{-7}$  mho)<sup>21</sup>. This small conductance increase during m.e.p.p.s. could be explained, for example, by a small amount of ACh in one packet or by a low ACh receptor density in the subsynaptic membrane. Patrick *et al.*<sup>14</sup> found 100 receptors per  $\mu\text{m}^2$  in non-innervated L6 myotubes, 0.3 to 1.4% of that found at adult end-plates in mammalian striated muscle<sup>22-24</sup>.

When a nerve bundle in contact with a myotube was stimulated with a suction electrode (tip diameter about 5  $\mu\text{m}$ , filled with saline), an e.p.p. was evoked (Fig. 4a). The nerve was stimulated about 50  $\mu\text{m}$  from the nerve-muscle contact. There was a relatively long latency (7 ms) between the nerve stimulation and the onset of the e.p.p. Although the e.p.p.s. sometimes failed (seven failures out of 22 consecutive stimulations), no discrete steps of e.p.p. amplitude were seen.

Addition of KCl (20 mM final concentration) did not change the m.e.p.p. frequency although it depolarised the muscle (Fig. 4b). This was the case even in nerve muscle pairs where e.p.p.s. could be evoked by nerve stimulation.

High tonicity causes a large increase in the m.e.p.p. frequency at adult synapses<sup>25</sup> but the addition of sucrose (400 mM) did not increase the m.e.p.p. frequency in this system. A similar

insensitivity to high KCl and high tonicity was found in newly formed *Xenopus* neuromuscular junctions *in vitro*<sup>3</sup>. In several cases lanthanum (about 0.1 mM) increased the m.e.p.p. frequency for a few minutes and then the m.e.p.p.s. disappeared. In most cases, however, lanthanum ions suppressed the m.e.p.p.s without any apparent stimulation. In adult frog neuromuscular junctions lanthanum ions increase the m.e.p.p. frequency for hours before the m.e.p.p.s. finally disappear<sup>26</sup>.

The electrophysiological properties of the synapses formed between the L6 cell line and spinal cord explants are similar to those reported in chick primary cultures<sup>1,2,4</sup>.

Thus a clonal muscle cell line adapted to continuous cell culture can make a synapse with a suitable nerve. Previous failures to detect functional synapses between L6 myotubes and the neuroblastoma cell line must have been due to the incompetence of the nerve. The availability of many independently derived nerve, glia and muscle cell lines<sup>27,28</sup> will make possible an investigation of the requirements of synapse formation.

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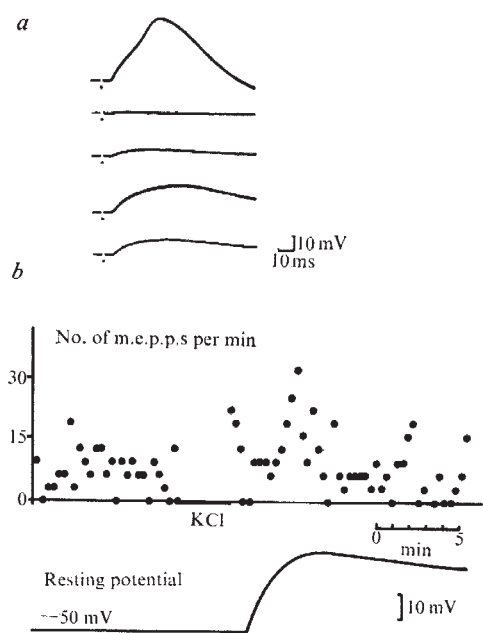


Fig. 4 a, e.p.p.s. evoked by stimulating the nerve bundle. Stimulus intensity was kept constant. The top record has a small regenerative potential superimposed on the e.p.p. b, The effect of KCl on m.e.p.p. frequency. A small amount of KCl (1 M) was added to the bath during the time indicated by a bar to a final concentration of 20 mM. The lower trace is the membrane potential of the muscle cell showing the time course of depolarisation.

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## Rapid reversal of internal $\text{Na}^+$ and $\text{K}^+$ contents of synaptosomes by ouabain

THERE is evidence that a number of drugs which have profound effects on the central nervous system (CNS) may do so by altering membrane bound ( $\text{Na}^+ + \text{K}^+$ )-dependent ATPase and/or  $\text{Na}^+$  pump activities. For example, ouabain which is an inhibitor of neuronal ( $\text{Na}^+ + \text{K}^+$ ) dependent ATPase<sup>1</sup> and  $\text{Na}^+$  pump activity<sup>2</sup>, can impair short term memory in small doses<sup>3</sup> and produce convulsions when larger doses are administered intraventricularly<sup>4</sup>.

It is generally considered that the synaptic region of the neurone is especially sensitive to the actions of drugs. In the case of drugs which modify ( $\text{Na}^+ + \text{K}^+$ )-dependent ATPase activity, it is interesting that this enzyme is found in abundance in synaptosomal preparations<sup>5</sup>. Moreover, synaptic terminals in the CNS, which are generally 1  $\mu\text{m}$  or less in diameter<sup>6</sup>, would have very small internal volumes so that alterations in  $\text{Na}^+$  pump activity might be expected to cause relatively rapid changes in the concentrations of  $\text{Na}^+$  and  $\text{K}^+$  inside the terminals. In contrast, the squid giant axon, with its much larger internal volume, and therefore larger reservoir of  $\text{K}^+$ , seems to be fairly resistant to  $\text{Na}^+$  pump inhibition insofar as it maintains its resting potential even after the  $\text{Na}^+$  pump has been inhibited by ouabain or metabolic inhibitors for over an hour<sup>2,7</sup>.

We have found that ouabain reversed the normal  $\text{Na}^+$  and  $\text{K}^+$  contents of synaptosomes within 1 min of exposure to the drug, indicating that presynaptic terminals might be particularly sensitive to drugs which influence  $\text{Na}^+$  pump activity.

Synaptosomes were prepared from the brains of 2 male Wistar rats (200–250 g) and isolated on sucrose density gradients as described previously<sup>8</sup>, before being finally resuspended with 0.5 ml of ice-cold 0.32 M sucrose. The synaptosomes were then incubated in media containing  $\text{NaCl}$ ,  $\text{KCl}$ ,  $\text{CaCl}_2$ ,  $\text{MgCl}_2$  and Tris-HCl and the effects of 1 mM ouabain on the  $\text{Na}^+$  and  $\text{K}^+$  contents determined as described in Fig. 1.

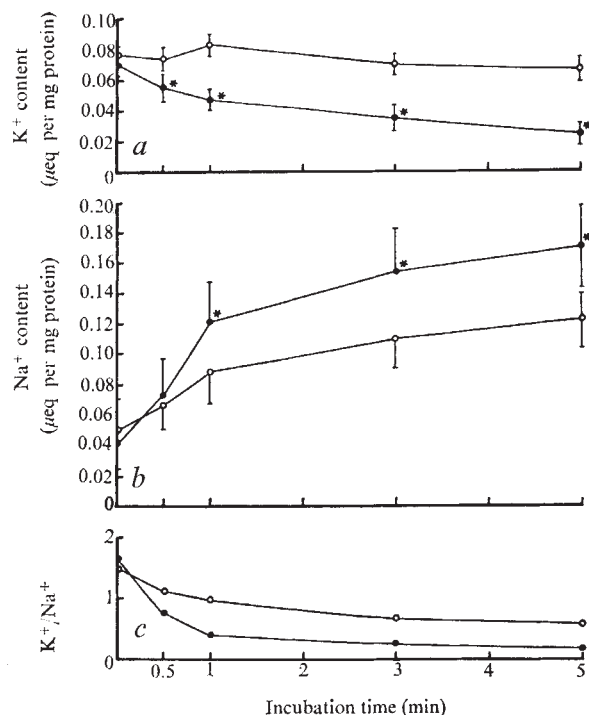
Figure 1a shows that 1 mM ouabain caused a significant reduction in synaptosomal  $\text{K}^+$  content (paired  $t$  test) at 30 s with a maximal effect by 1 min. Synaptosomes gained  $\text{Na}^+$  during incubation (Fig. 1b) but ouabain increased further the  $\text{Na}^+$  content after 1 min (paired  $t$  test) with a maximal effect achieved after 3 min. At time zero, the  $\text{K}^+/\text{Na}^+$  ratio was 1.51 for the control synaptosomes (Fig. 1c) which compares favourably with the value of 1.78 found previously for synaptosomes immediately after isolation on a discontinuous sucrose density gradient<sup>12</sup>. During incubation for 1 min, this ratio decreased somewhat to 0.93 for the controls but ouabain completely reversed the  $\text{K}^+/\text{Na}^+$  ratio to 0.39 by 1 min.

These results clearly indicate that ouabain can very quickly reverse the  $\text{Na}^+$  and  $\text{K}^+$  contents of synaptosomes. If, as much evidence suggests, synaptosomes are functionally intact presynaptic nerve terminals<sup>13–15</sup>, and if one can extrapolate from these *in vitro* experiments to the *in vivo* situation, it would seem that alterations of synaptic  $\text{Na}^+$  pump activity might very quickly change the internal  $\text{Na}^+$  and  $\text{K}^+$  contents of synaptic terminals, possibly because of the very small internal volumes of the terminals. This rapid change in  $\text{Na}^+$  and  $\text{K}^+$  content could in turn affect the resting membrane potential<sup>7</sup>, the generation of action potentials<sup>7</sup>, the release and reuptake of neurotransmitters<sup>6,16</sup> and even protein synthesis<sup>17,18</sup> in the presynaptic terminal. Thus, one might expect synaptic transmission to be particularly susceptible to alterations in neuronal  $\text{Na}^+$  pump activity.

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**Fig. 1** Time course for the effect of ouabain on the  $\text{Na}^+$  and  $\text{K}^+$  contents of synaptosomes. Incubation medium (2 ml) containing 120 mM  $\text{NaCl}$ , 3 mM  $\text{KCl}$ , 3 mM  $\text{MgCl}_2$ , 2 mM  $\text{CaCl}_2$ , 20 mM Tris-HCl (pH 7.4) and sometimes 1 mM ouabain were prewarmed to 37° C under air in a 10 ml conical flask in a shaking incubator. To avoid any effects of ouabain on glucose uptake which might indirectly influence synaptosomal  $\text{Na}^+$  pump activity, glucose was omitted from the incubation medium. At zero time, 0.10 ml of synaptosomal suspension was added and the incubation continued; the reaction was stopped by removing and filtering 0.5 ml of medium on a Millipore filter (0.45  $\mu\text{m}$  pore size and 2.5 cm diameter) and washing with 10 ml, ice-cold medium containing 260 mM sucrose, 3 mM  $\text{MgCl}_2$ , 2 mM  $\text{CaCl}_2$  and 20 mM Tris-HCl (pH 7.4). This washing procedure, which took approximately 20 s, did not seem to rupture the synaptosomes<sup>9</sup>. A 1.5 cm diameter disk containing the synaptosomes was cut out from the filter and  $\text{Na}^+$  and  $\text{K}^+$  were eluted overnight with 0.6 ml of 15 meq  $\text{l}^{-1}$  lithium diluent solution (Instrumentation Laboratory, Inc., lithium nitrate standard). This hypotonic treatment releases the  $\text{Na}^+$  and  $\text{K}^+$  contained in osmotically sensitive compartments of the synaptosomes. The  $\text{Na}^+$  and  $\text{K}^+$  contents of the diluent were determined with an IL model 143 flame photometer and the values corrected for the  $\text{Na}^+$  and  $\text{K}^+$  eluted from the filter itself by subtracting the amount of  $\text{Na}^+$  and  $\text{K}^+$  released into diluent from filters which had been treated as above except that no synaptosomes were present in the incubation medium.  $\text{Na}^+$  contamination due to trapping of incubation medium in the extracellular space of the synaptosomes on the filters was determined in the following manner. Synaptosomes were incubated as before but 0.01 ml of  $^{35}\text{S}$ -sulphate (1 mCi  $\text{ml}^{-1}$ ) as an extracellular marker<sup>6</sup> was added immediately before filtering and washing. The radioactivity in the filter was determined by scintillation counting and compared with the radioactivity present originally in 0.01 ml of the  $^{35}\text{S}$ -sulphate solution to give the percentage contamination of the filter and synaptosomes by incubation medium. This estimate was performed for various concentrations of synaptosomal protein including a blank which contained no synaptosomes. The latter value was subtracted from the values obtained with synaptosomes present to give the amount of contamination in the extracellular space and these values were used to calculate the percentage contamination by incubation medium. The extrasynaptosomal  $\text{Na}^+$  contamination from the incubation medium was calculated and subtracted from the total  $\text{Na}^+$  content of the synaptosomes on the filter to give the actual  $\text{Na}^+$  content of the synaptosomes. Contamination by extracellular  $\text{K}^+$  was not corrected because of the small amounts of  $\text{K}^+$  present in the original incubation media. The final ion contents were expressed as  $\mu\text{eq}$  ion per mg synaptosomal protein. Protein was determined by the Hartree<sup>10</sup> modification of the method of Lowry *et al.*<sup>11</sup>.  $^{35}\text{S}$ -sulphate was supplied by New England Nuclear Corp., Ltd., Montreal and ouabain by the Sigma Chemical Co., St. Louis. a,  $\text{K}^+$  content; b,  $\text{Na}^+$  content; c, ratio of  $\text{K}^+/\text{Na}^+$ ; ○, control; ●, 1 mM ouabain. Each point represents the mean  $\pm$  s.e. of 12 determinations (six experiments). \* Ouabain treatment is significantly different from the control ( $P < 0.05$ , paired  $t$  test).

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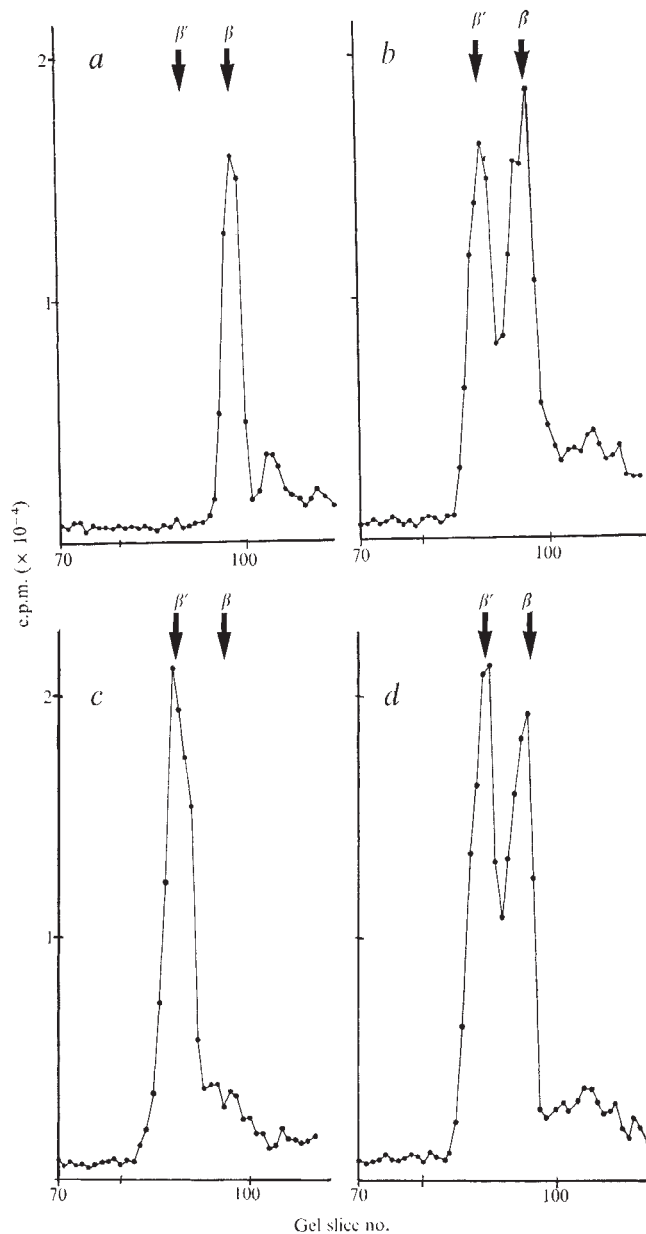
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## Coordinate and differential *in vitro* syntheses of two RNA polymerase subunits

THE  $\beta$  subunit of *Escherichia coli* RNA polymerase—one of three subunit types ( $\alpha$ ,  $\beta$ ,  $\beta'$ ) in the enzyme core structure—is specified by a genetic locus, *rif* (ref. 1). Specialised transducing phages which carry the *rif* region have been isolated<sup>2,3</sup>. If DNA is extracted from these phages, it can be used to direct the synthesis of the  $\beta$  subunit *in vitro* in a coupled transcription/translation system<sup>4,5</sup>. It has been suggested that other RNA polymerase structural genes may also be in this region of the chromosome<sup>6</sup>. Recent genetic evidence shows that the  $\beta$  and  $\beta'$  subunits are produced from a single transcriptional unit *in vivo*<sup>7</sup>. DNA extracted from three independently isolated *rif* transducing phages however, failed to direct the synthesis of any detectable  $\beta'$  molecules *in vitro* in the coupled systems previously described<sup>4,5</sup> (Fig. 1a). Here I describe a coupled system which synthesises  $\beta$  and  $\beta'$  *in vitro* with equal efficiency from *rif* transducing phage DNA.

The new method involves the use of modifications in the preparation of bacterial extracts (S30s) similar to those developed by Wilcox *et al.* who used them to maximise the synthesis of arabinose isomerase *in vitro* using  $\lambda$ h<sub>80</sub>dara template DNA<sup>8</sup>. The data presented in Fig. 1 provide strong evidence that both  $\beta$  and  $\beta'$  subunits can be synthesised in the modified system, using  $\phi$ 80d,*rif*<sup>7</sup> DNA as a template. Two newly-synthesised proteins migrate upon electrophoresis in sodium dodecylsulphate polyacrylamide gels to positions coincident with those of marker bands for  $\beta$  and  $\beta'$ . These proteins are specifically precipitated by serum prepared against highly purified *E. coli* RNA polymerase (Fig. 1). Thus these proteins are antigenically related to purified RNA polymerase, and have apparently identical molecular weights to those of the  $\beta$  and  $\beta'$  subunits.

If the proteins made *in vitro* are indeed RNA polymerase subunits, then DNA-containing mutations which alter polymerase structural gene expression *in vivo* should also affect the synthesis of the proteins *in vitro*. We have previously described a class of mutations (*rif*<sup>o</sup> mutations) which are candidates for  $\beta$  structural gene mutations<sup>9</sup>. The *rif*<sup>o</sup> mutations



**Fig. 1** Cell free protein synthesising systems containing type 1 or type 2 S30s were run as previously described<sup>4</sup> with the addition of 15 mg ml<sup>-1</sup> of polyethylene glycol and 0.2  $\mu$ mol disphosphopyridine nucleotide. 0.1 ml systems contained 0.3 mCi of <sup>3</sup>H-L-leucine, specific activity 60 Ci mmol<sup>-1</sup>, 10  $\mu$ g of template DNA, and either 2 nmol *E. coli* transfer RNA from a non-suppressing (*su*<sup>-</sup>) strain or 2 nmol of tRNA enriched for the suppressor *SuIII* tRNA<sup>10</sup>. After incubation, the systems were diluted to 0.4 ml with buffer having the same salt composition as the cell free system<sup>4</sup>, mixed thoroughly, and centrifuged at 4°C at 40,000g for 15 min. The supernatant was decanted into a tube containing 20  $\mu$ l of antiserum prepared against highly purified RNA polymerase. This serum is highly specific for the participation of RNA polymerase and its subunits (S.A., unpublished). After 12 h at 4°C the precipitate was collected by centrifugation at 40,000g for 30 min. The pellet was resuspended in 50  $\mu$ l of sample buffer<sup>4</sup>, and was run on sodium dodecylsulphate polyacrylamide gels as previously described<sup>4</sup>, except that 2 mm thick slab gels<sup>14</sup> were substituted for the tubular ones. The gels were stained, sliced, and counted for radioactivity as previously described<sup>4</sup>. The arrows show the position of the stained marker bands for  $\beta$  and  $\beta'$ . a, Type 1 S30,  $\phi$ 80d,*rif*<sup>o</sup> DNA; b, type 2 S30,  $\phi$ 80d,*rif*<sup>o</sup> DNA; c, type 2 S30,  $\phi$ 80d,*rif*<sup>o</sup>-B115 DNA; d, type 2 S30 plus *SuIII* enriched tRNA,  $\phi$ 80d,*rif*<sup>o</sup>-B115 DNA.



**Table 1** The synthesis of arabinose isomerase and  $\beta$  galactosidase *in vitro* using type 1 and type 2 S30 cell extracts

| DNA template                  | Arabinose isomerase* |            | $\beta$ -galactosidase* |            |
|-------------------------------|----------------------|------------|-------------------------|------------|
|                               | Type 1 S30           | Type 2 S30 | Type 1 S30              | Type 2 S30 |
| $\lambda$ h <sub>80dara</sub> | <0.04                | 3.2        | <0.01                   | <0.01      |
| $\lambda$ h <sub>80dlac</sub> | <0.04                | <0.04      | 12                      | 22         |

Type 1 S30 cell extracts were prepared as described<sup>11</sup> by lysis in an Aminoc French Pressure Cell at 5,000 pounds per square inch pressure. Cell extracts prepared by alumina grinding<sup>4</sup>, were also used and had identical properties to type 1 S30s. Type 2 S30s were prepared as described<sup>8</sup> but with French Cell pressures further reduced, so that 30% of the bacteria remain unbroken (600–1,000 pounds per square inch). Both types of S30 were active for protein synthesis as shown by their ability to promote  $\beta$ -galactosidase synthesis from  $\lambda$ h<sub>80dlac</sub> template DNA (Table 1). The protein synthesis mixtures<sup>8</sup> were incubated for 80 min at 35.5°C with  $\mu$ g ml<sup>-1</sup> of template DNA. Arabinose isomerase activity was dependent on *de novo* synthesis of the regulatory product directed by the *ara* C gene<sup>8</sup>. The C gene product can also be made using type 1 S30's (ref. 13).

\*Arabinose isomerase assays<sup>8</sup> and  $\beta$ -galactosidase assays<sup>9</sup> are expressed as  $\mu$ mol of substrate converted h<sup>-1</sup> ml<sup>-1</sup> of *in vitro* system; 1 ml assay mixtures contained 0.1 ml of the *in vitro* system.

eliminate the expression of the rifampicin sensitivity phenotype, which is known to be associated with the  $\beta$  structural gene<sup>1</sup>. I have transferred a *rif*<sup>o</sup> mutation of the chain terminating class, *rif*<sup>o</sup>-B115 *amber*, from the host chromosome to the  $\phi$ 80d<sub>7</sub>*rif*<sup>r</sup> transducing phage by recombination (Austin, in preparation). DNA extracted from the recombinant phage,  $\phi$ 80d<sub>7</sub>*rif*<sup>o</sup>-B112, produces no  $\beta$  peak *in vitro* but still directs  $\beta'$  synthesis (Fig. 1c). If purified *E. coli* transfer RNA, enriched for mutant *suIII* tRNA is added to the system<sup>10</sup> the synthesis of the  $\beta$  protein is restored (Fig. 1d). Thus *rif*<sup>o</sup>-B115 is a suppressible nonsense mutation which eliminates the expression of the  $\beta$  gene. As it has no apparent effect on  $\beta'$ , a gene distal to the  $\beta$  gene in the same operon<sup>7</sup>, *rif*<sup>o</sup>-B115 is probably a  $\beta$  structural gene mutation.

The difference in results obtained with the *in vitro* system by changing the type of S30 used is notable. The use of the modified (Type 2) S30 is, within the limits of detection of my assays, an absolute requirement for the synthesis of  $\beta'$  from  $\phi$ 80d<sub>7</sub>*rif*<sup>r</sup> DNA, and of arabinose isomerase from  $\phi$ 80d<sub>ara</sub> DNA (Fig. 1, Table 1). The original, however, (Type 1), S30s (ref. 11) suffice for the synthesis of  $\beta$  subunit from  $\phi$ 80d<sub>7</sub>*rsf*<sup>r</sup> (Fig. 1). A second product of the *ara* operon, ribulokinase, can also be made efficiently using type 1 S30s (ref. 12). Thus the *in vitro* system can show a striking discrimination between two products which at least *in vivo*, are the products of a single messenger RNA. Both the *rif* and *ara* operon show this effect. This suggests that the effect may have some general relevance to the production of proteins from polycistronic messengers. I am now investigating whether the discrimination occurs at the level of transcription or translation. The present system should provide an assay for attempts to purify a discriminating agent from S30 extracts.

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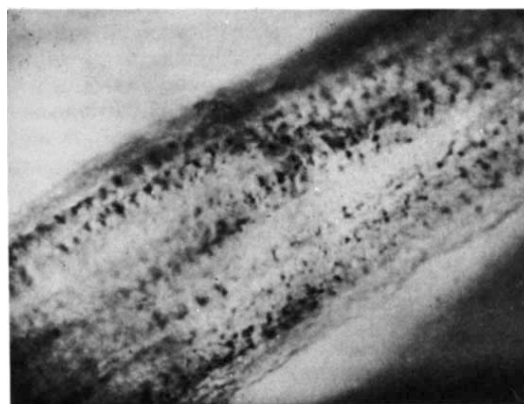
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## Alteration of melanocytes by DNA in White Plymouth Rock chickens

SINCE 1944 when it was first demonstrated that the pneumococcal transforming principle was DNA<sup>1</sup>, several investigators have reported evidence suggesting genetic alteration of warm-blooded animal cells by DNA-mediated transformations<sup>2–7</sup>. Attempts to transform the plumage coloration characters in White Plymouth Rock embryos have been unsuccessful<sup>8</sup>. The genetically lethal melanocytes in the White Plymouth Rock chicken do not appear abundantly or thrive for long in the feather germ. They degenerate before they can deposit a significant number of melanin granules in the feather-forming cells<sup>9</sup>. Using nucleoprotein from the Harco chicken, a standard cross between the Barred Plymouth Rock hen and the Rhode Island Red rooster, I have extended the life span of the melanocytes in White Plymouth Rock embryos.

Nucleoprotein was extracted from about 1 g (wet weight) of feather anlage, from 10-d-old Harco chicken embryos. The tissue was suspended in a solution of 0.25 M sucrose (pH 7.4) and 1 mM EDTA at a 1:9 (w/v) ratio of tissue to sucrose. The mixture was homogenised in a tissue grinder with an inhibitor of DNase (NaF, 0.044 M). Nucleoprotein was extracted with 2 M sodium chloride as described by Zamenhof<sup>10</sup>, except that the mixture was left at 4°C for only 2 h to avoid damage to DNA. The nucleoprotein was suspended in 4 ml of normal saline and divided into two parts: one part was left untreated and to the second part, crystalline pancreatic DNase (0.1 mg ml<sup>-2</sup>) and MgCl<sub>2</sub>·6H<sub>2</sub>O (5 mM) were added. The preparations were incubated at 25°C for 1.5 h and then 0.04 ml was injected into small extra-embryonic veins of 5-d-old



**Fig. 1** Light micrograph of melanin granules in the feather anlage from the head of a 14-d-old White Plymouth Rock embryo, which was injected with DNA on the day 5 of incubation ( $\times 100$ ).

White Plymouth Rock embryos. By this time the melanoblasts have migrated from the neural crest into the skin<sup>11</sup>.

Twelve of the nineteen embryos injected with nucleoprotein survived beyond day 14 of incubation. Starting from this time and continuing until birth, nine of these embryos were examined at 2-d-intervals. The host embryo's melanocytes would have degenerated by this time if left undisturbed. The remaining three embryos were allowed to hatch. Patches of pigmented feathers were found on five of the embryos and on the juvenile down of one newborn chick (Fig. 1). Their coats were otherwise white.

The active component in the transforming material seems to be DNA because no DNase-treated preparation induced pigmented feathers. Thus treatment of White Plymouth Rock embryos with DNA carrying a marker for full-lived melanocytes resulted in the development of pigmented feathers in six out of twelve cases.

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## *In vitro* maturation of precursors of 5S ribosomal RNA from *Bacillus subtilis*

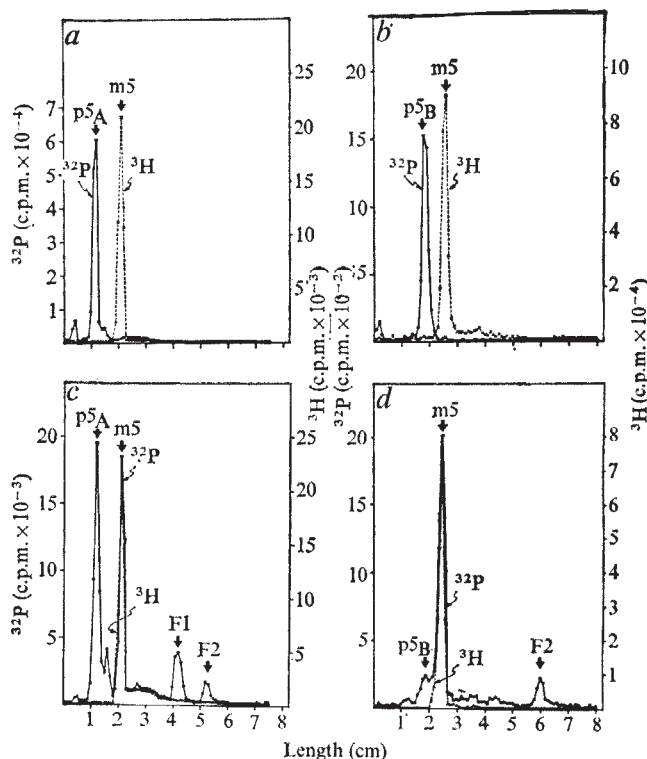
THE ribosomal RNA (rRNA) molecules both of prokaryotes and of eukaryotes are not the immediate products of DNA transcription. Rather, precursors of the mature RNA species undergo a series of nucleotide modifications and/or cleavage events<sup>1,2</sup>. Only limited information is available concerning the mechanics of and rationale for post-transcriptional tailoring of RNA because of the large sizes of the precursor molecules and consequent difficulty in evaluating their nucleotide sequences.

By virtue of its small size (about 120 nucleotides), 5S rRNA is convenient for study but its maturation is not always as complex as that affecting the high molecular weight rRNA molecules (16S and 23S rRNA, in prokaryotes). In *Escherichia coli*, for example, the precursor of 5S rRNA (p5) is converted to the mature molecule (m5) by an exonuclease<sup>3,4</sup> rather than a specific endonuclease, which probably is responsible for maturation of 16S and 23S rRNA. In *Bacillus subtilis*, however, metabolism of 5S rRNA apparently mimics that of the larger rRNA molecules<sup>5</sup>. In the absence of protein synthesis (presence of chloramphenicol) *B. subtilis* accumulates two precursors of 5S rRNA, designated p5<sub>A</sub> (180 nucleotides) and p5<sub>B</sub> (150 nucleotides). Both are converted independently to m5 rRNA, which in this organism is composed of 118 nucleotides. We here report that maturation of p5<sub>A</sub> and p5<sub>B</sub> is effected *in vitro* by a partially purified, specific cleavage enzyme, which we denote as RNase M5.

Incubation of purified <sup>32</sup>P-labelled p5<sub>A</sub> and p5<sub>B</sub> with partially purified RNase M5 from *B. subtilis* yields several products. Figure 1 displays polyacrylamide gel electrophoretograms of the

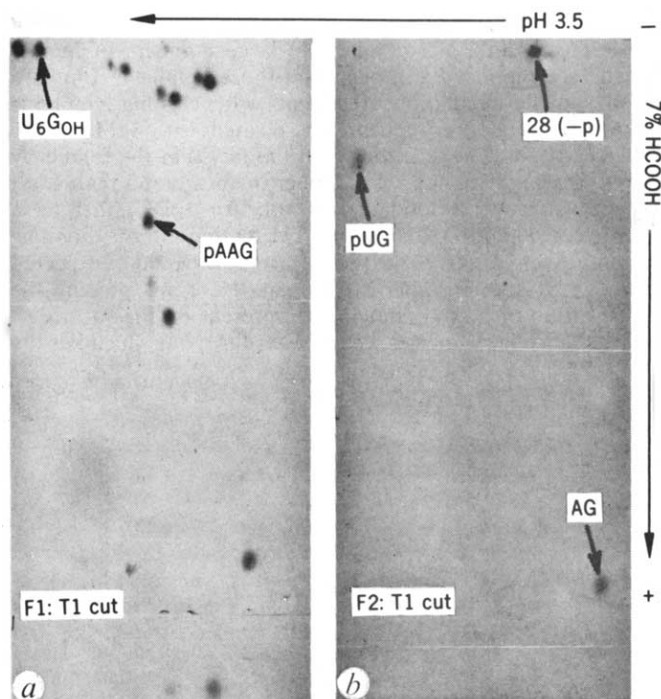
purified substrates (Fig. 1a and b) and the products of the cleavage reaction (Fig. 1c and d). The p5<sub>A</sub> molecule yields three products, one coincident with added <sup>3</sup>H-labelled m5 rRNA in electrophoretic mobility, a second about forty nucleotides in length (designated F1) and a third composed of 22 nucleotides (designated F2). Two-dimensional electrophoretic analysis (data not shown) of T1 and pancreatic RNase digests of the 5S material generated during the reaction shows that it possesses the same 5' (p-U-U-U-G-) and 3' (-A-A-G-C<sub>OH</sub>) termini as 5S rRNA obtained from ribosomes; the molecules are identical. The products of p5<sub>B</sub> cleavage include one component identical in size and structure to the added <sup>3</sup>H-m5 rRNA and a second fragment having the same electrophoretic mobility and, as it proved, the same detailed structure as F2. Also derived from p5<sub>B</sub> is a component which is heterogeneous in size (about 8–15 nucleotides) which we have not characterised in detail.

Evaluation of the T1 RNase digestion products of the fragments released during the reaction defines their origin in the precursors (Fig. 2). Of particular note is that F1 contains -U-U-U-U-U-G<sub>OH</sub> (Fig. 2a), the 3' terminus of F1 and of p5<sub>A</sub> (M. Rosenberg, unpublished), whereas F2 yields p-U-G- (Fig. 2b), the 5' terminus of both precursors. F1 therefore must



**Fig. 1** Conversion of p5<sub>A</sub> and p5<sub>B</sub> to m5 rRNA. <sup>32</sup>P-labelled p5<sub>A</sub> or p5<sub>B</sub> prepared as described previously<sup>6</sup> were incubated at 37° C for 15 min in the presence or absence of Sup 2 RNase M5 from *B. subtilis* 168 in 0.01 M Tris-HCl, pH 7.5, 0.05 M NH<sub>4</sub>Cl, 0.005 M MgCl<sub>2</sub>. Reaction was halted by addition of sodium dodecyl sulphate to 1% and ethylene diamine tetraacetic acid to 0.01 M, then <sup>3</sup>H-labelled m5 rRNA was added as a size marker and products were resolved by electrophoresis through 8% polyacrylamide gels<sup>5</sup>. Materials migrating in gels between the m5 and F1 regions are products of nonspecific degradation. a, <sup>32</sup>P-p5<sub>A</sub> plus <sup>3</sup>H-m5 rRNA (no enzyme); b, <sup>32</sup>P-p5<sub>B</sub> plus <sup>3</sup>H-m5 rRNA (no enzyme); c, <sup>32</sup>P-p5<sub>A</sub> incubated with Sup 2 RNase M5 preparation; d, <sup>32</sup>P-p5<sub>B</sub> incubated with Sup 2 RNase M5 preparation. Sup 2 was prepared as follows: 2 gm of *B. subtilis* 168 cell paste was resuspended in 4 ml buffer SB (0.05 M Tris-HCl, pH 7.5, 0.06 M NH<sub>4</sub>Cl, 0.01 M MgCl<sub>2</sub>, 0.0001 M EDTA-Na<sub>2</sub>, 0.0001 M dithiothreitol, 5% glycerol) containing 10 µg ml<sup>-1</sup> DNase. Cells were disrupted with a French press, and the lysate was diluted twofold with buffer SB and cleared by centrifugation at 20,000 r.p.m. (Sorvall SS-34 rotor) for 20 min. The supernatant is Sup 0. Ribosomes were pelleted from Sup 0 by centrifugation at 40,000 r.p.m. (Spinco 40 rotor) for 2 h, resuspended in SB, adjusted to contain 0.2 M NH<sub>4</sub>Cl, and again pelleted. The supernatant is Sup 2.

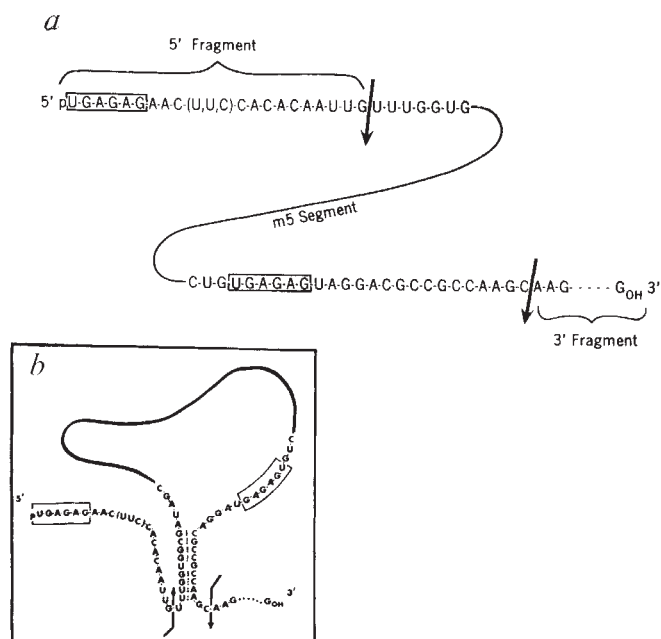




**Fig. 2** Two-dimensional electrophoresis of T1 RNase digests of F1 and F2. Fragments designated F1 and F2 were generated from p5<sub>A</sub> by incubation with Sup 2 and resolved by gel electrophoresis as described in Fig. 1. Following elution from gels, the fragments were digested completely by T1 RNase and resulting oligonucleotides were resolved by two-dimensional electrophoresis on cellulose acetate at pH 3.5, followed by diethylaminoethyl paper at pH 1.9, as detailed by Sanger and Brownlee<sup>6</sup>. *a*, RNase T1 digest of *in vitro* F1; *b*, RNase T1 digest of *in vitro* F2.

represent the 3' and F2 the 5' terminal, precursor-specific segments of p5<sub>A</sub>. The scission events release 5'-phosphoryl and 3'-hydroxyl termini. Recovery of these fragments establishes that RNase M5 is a specific endonuclease.

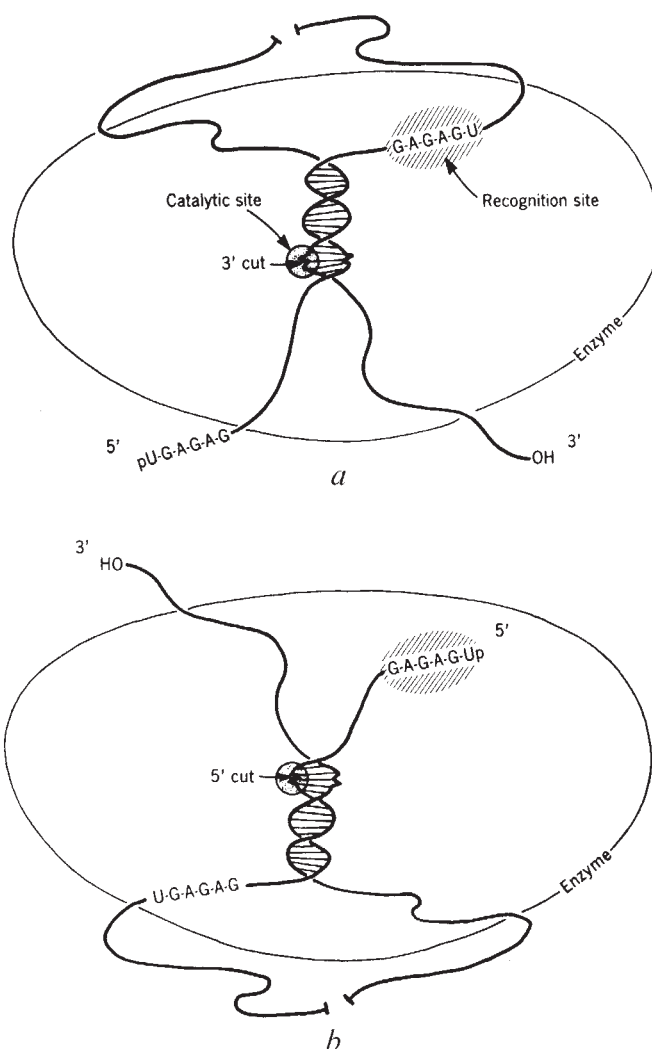
We have not yet rigorously purified RNase M5 of *B. subtilis*, but substantial information regarding the enzyme is available (M.L.S., and N.R.P., unpublished). Apparently, a single catalytic activity is responsible for removing both the 5' and 3'



**Fig. 3** Partial nucleotide sequence of p5<sub>A</sub> of *B. subtilis*; *a*, as a linear molecule and, *b*, with the 5' and 3' terminal regions of the m5 portion of the precursor juxtaposed in an antiparallel, duplex stalk.

terminal, precursor-specific segments of p5<sub>A</sub> and p5<sub>B</sub>. This is suggested by our inability to separate 5' and 3' cleavage activities by chromatography on DEAE cellulose or by zonal sedimentation through sucrose gradients. Moreover, the 5' and 3' cleavage activities display identical inactivation kinetics. RNase M5 is a large protein, 5–6S in size, corresponding to a molecular weight of  $1 \times 10^5$ – $1.5 \times 10^5$ . Maximal activity requires as well the presence in reactions of at least one relatively low molecular weight component, possibly a ribosomal protein. As a single enzyme removes both the 5' and 3' terminal precursor-specific sequences, it is likely that single catalytic and recognition sites are involved in interaction with the substrate molecules. In principle, therefore, it is possible to suggest features of the precursor molecules which interact with RNase M5 by identifying structural similarities in the vicinities of the two endonucleolytic cuts.

The nucleotide sequences of the precursor and mature 5S rRNA molecules from *B. subtilis* are almost completely determined (M.L.S. and N.R.P., and S. M. Weissman *et al.*, unpublished). Relevant portions of the p5<sub>A</sub> molecule are shown in Fig. 3. The 5' and 3' terminal regions of the m5 portion of the precursor are juxtaposed in an antiparallel, duplex stalk. This feature is common to all 5S rRNA molecules so far examined and probably exists both in solution and in the ribosome. The points at which endonucleolytic cleavage occur are indicated. It is noteworthy that the cuts at the 5' and 3' termini of the molecule are spatially quite near to each other when the hydrogen-bonded stalk is constructed, and in fact the points of scission probably are within a duplex region. It seems unlikely, however,



**Fig. 4** Alternative models for the interaction of p5<sub>A</sub> with RNase M5 (for an explanation of *a* and *b* see text).

that both endonucleolytic cuts are effected at the same time because the catalytic site on the enzyme surface very probably must consider the polarity of the phosphodiester bond being acted upon. The two bonds to be cleaved would present opposite polarities upon simultaneous inspection by a catalytic site.

It is evident from Fig. 3 that the sequences in the immediate vicinities of the endonucleolytic cuts are dissimilar; RNase M5 is unlikely to act at independent substrate sites on this basis. The sequence -U-G-A-G-A-G-, however, lies 16 nucleotides 5' removed from both points of cleavage. One of these sequences is at the 5' terminus of a precursor-specific segment whereas the second lies within the body of the m5 portion of the molecule. The probability of random assignment of two identical hexameric sequences equidistant from two given points (the cleaved sites) is about  $2.4 \times 10^{-4}$ , so we suppose that this sequence constitutes at least part of that feature of the precursors which is recognised by the cleavage enzyme. If both the duplex stalk and the U-G-A-G-A-G sequence are considered to be involved in orientation of the substrate, then structural symmetry about the bonds to be cleaved is apparent, as is diagrammatically depicted in Fig. 4. Figure 4a positions the U-G-A-G-A-G in the body of the m5 sequence at a 'recognition site' on the enzyme surface, and at the 'catalytic site' is placed the phosphodiester bond whose cleavage will release the 3' terminal, precursor-specific segment.

Alternately (or following release of the fragment) (Fig. 4b), the substrate could be inverted and rotated about the helical axis of the stalk to place the 5' terminal U-G-A-G-A-G into the 'recognition site'; this could place at the 'catalytic site' the phosphodiester bond whose hydrolysis releases the 5' terminal precursor-specific segment. Both U-G-A-G-A-G sequences are aligned at the recognition site on the enzyme surface with the same chemical polarity and, similarly, the phosphodiester bonds to be cleaved are positioned at the catalytic site with the same polarity. The structural simplicity of the 5S rRNA molecule thus permits tentative identification of features within one type of polynucleotide which may interact in a highly specific fashion with a protein. The actual involvement of the proposed recognition-alignment elements and their relationships to the phosphodiester substrate bonds during the cleavage reaction are experimentally testable.

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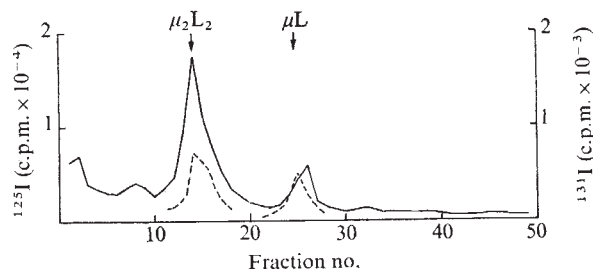
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## Candidate for immunoglobulin D present on murine B lymphocytes

ALTHOUGH the original discovery of human IgD is almost ten years old<sup>1</sup>, it is only very recently that a possible function for this class of immunoglobulin has been suggested. Present in

normal human serum in very small amounts<sup>2</sup>, IgD is found on the surface membrane of relatively large numbers of human peripheral lymphocytes, in particular those obtained from cord blood and the circulation of patients with chronic lymphatic leukaemia<sup>3-7</sup>. IgD is frequently associated with IgM on the same cell<sup>5-7</sup> and this, together with the fact that the frequency of IgD-bearing lymphocytes is higher in cord blood than adult blood<sup>4,5</sup>, suggests a fundamental role for IgD, either as a primitive recognition unit or for regulation of the immune response. Were this to be so, then clearly IgD would be expected to occur in species other than man. Here we present the identification of an immunoglobulin present on the surface of mouse splenic lymphocytes which is not IgM, but which has the predicted characteristics of IgD.



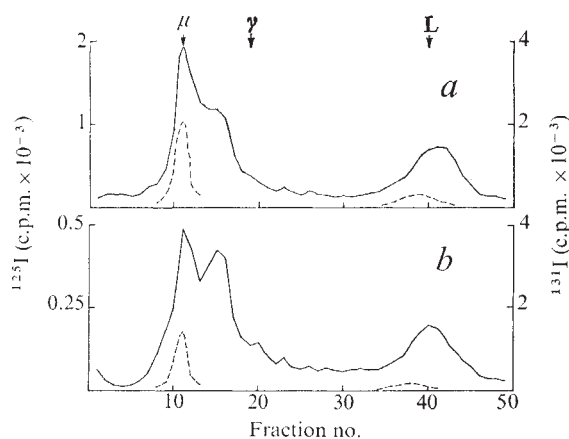
**Fig. 1** Surface immunoglobulin of murine splenic lymphocytes. Spleen cell suspensions of 4-6-week-old female CBA mice were labelled with <sup>125</sup>I by the lactoperoxidase catalysed procedure<sup>8</sup>, washed once in phosphate-buffered saline (PBS) and then lysed for 10 min at 0° C in 1% (w/v) Nonidet P40 in PBS. The centrifuged lysate (4,000g × 10 min), containing 95% of the total TCA-precipitable radioactivity, was dialysed against 1% (w/v) Nonidet P40-PBS in the cold. Surface-labelled immunoglobulin was precipitated by the addition of a polyspecific rabbit anti-mouse Ig serum (10 µl) followed by goat anti-rabbit IgG (100 µl). The precipitate was washed three times with ice cold 0.5% (w/v) Nonidet P40 in PBS, twice with ice cold PBS, and then dissolved in 4% (w/v) sodium dodecyl sulphate (0.1 M iodoacetamide) 0.05 sodium phosphate, pH 7.0, by heating at 100° C for 5 min. A sample of mouse myeloma MOPC 104E IgM was labelled with <sup>131</sup>I (ref. 16), partially reduced to  $\mu_2L_2$  and  $\mu L$  by treatment with 1 mM dithiothreitol for 60 min at room temperature, alkylated with 10 mM iodoacetamide, and then added to the isolated <sup>125</sup>I-cell surface Ig to serve as an internal marker for the gel analysis (4.25% (w/v) polyacrylamide gel containing 0.1% (w/v) sodium dodecyl sulphate)<sup>9</sup>. Following electrophoresis, the gel was sliced into 1 mm segments and radioactivity determined. Values were corrected for cross channel spill, and plotted with the top of the gel on the left hand side of the figure. —, <sup>125</sup>I incorporated into cell surface Ig; ---, <sup>131</sup>I-labelled internal marker.

Spleen cell suspensions containing more than 95% viable cells (trypan blue exclusion) were labelled with <sup>125</sup>I by the lactoperoxidase-catalysed procedure<sup>8</sup>, and the surface immunoglobulin was prepared and analysed on sodium dodecyl sulphate (SDS) gels<sup>9</sup> with addition of an internal marker (<sup>131</sup>I-labelled IgM<sup>10</sup> partially reduced to  $\mu_2L_2$  and  $\mu L$ ). As has been previously described<sup>11,12</sup>, we found a major portion of radioactive cell surface immunoglobulin in that part of the gel corresponding to the IgMs ( $\mu_2L_2$ ) subunit of IgM (Fig. 1). In addition, however, there was a significant radioactive peak running very slightly in advance of the  $\mu L$  subunit, but which contained heavy and light chains on reduction (see Fig. 2) and must therefore be an HL subunit.

Following reduction and alkylation both the  $\mu_2L_2$  and HL subunits were shown to contain two distinct species of heavy chain, there being one component with the mobility (and therefore size) of the internal <sup>131</sup>I- $\mu$  chain marker, and another which migrated faster than  $\mu$  chain, but slower than  $\gamma$  chain (Fig. 2). The proportion of radioactivity found in the smaller (faster migrating) heavy chain was lower in the sample of reduced <sup>125</sup>I-surface  $\mu_2L_2$  (Fig. 2a) than the reduced <sup>125</sup>I-surface HL (Fig. 2b).

We discount the possibility that the heterogeneity of surface





**Fig. 2** Heavy chain heterogeneity of surface immunoglobulin of murine splenic lymphocytes. Sections of the gel shown in Fig. 1 containing the  $H_2L_2$  and HL components were eluted with 2% (w/v) sodium dodecyl sulphate (2mM dithiothreitol) 0.05 M sodium phosphate, pH 7.0. The eluate was heated for 15 min at 100°C in order to ensure reduction of all disulphide bridges, and then alkylated by addition of iodoacetamide to 10 mM. The resulting material was then applied to 10% (w/v) polyacrylamide gels containing sodium dodecyl sulphate to resolve the heavy chain components. In these conditions the  $^{131}\text{I}$ - $\mu_2L_2$  and  $\mu L$  originally added as internal markers for the first gel are also extracted and reduced, thereby providing internal markers for  $\mu$  and L chains in the second separation in the 10% acrylamide gels. The gels were fractionated, counted and plotted as given in the legend to Fig. 2. The position of  $\gamma$  chains was assessed by running a mixture of totally reduced  $^{131}\text{I}$ -labelled mouse IgM (MOPC 104E) and IgG2a (Adj.PC5) on a parallel gel. *a*, Reduced, surface labelled  $H_2L_2$ ; *b*, reduced, surface labelled HL. —,  $^{125}\text{I}$  incorporated into cell-surface Ig; ---,  $^{131}\text{I}$ -labelled internal marker.

Ig results from the presence of T-lymphocytes in the spleen cell suspension as similar results were obtained when spleen cells from nude mice were analysed by the same techniques. In addition, peripheral T-lymphocytes purified by passage of spleen cells through nylon wool columns<sup>13</sup> did not contain detectable amounts of surface immunoglobulin using the methods described above.

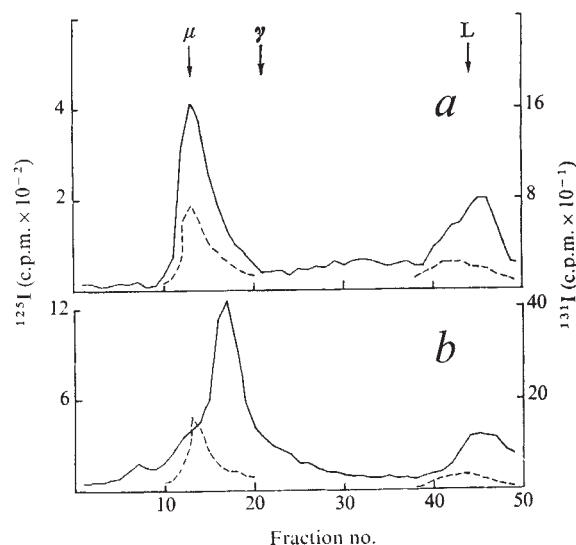
Reconstruction experiments were performed to check for degradation of  $\mu$  chain-containing material during the preparation of labelled surface Ig. Both  $^{125}\text{I}$ -labelled  $\mu_2L_2$  and  $\mu L$ , prepared by partial reduction of MOPC 104E IgM, could be recovered unchanged when added to spleen cells and then carried through the procedures outlined above for isolation of cell surface immunoglobulin. The HL subunits found on the surface of splenic lymphocytes, therefore, do not result from reductive depolymerisation of  $\mu_2L_2$  during isolation. Reduction of the recovered  $^{125}\text{I}$ - $\mu_2L_2$  and  $^{125}\text{I}$ - $\mu L$  yielded heavy chains entirely corresponding in size to untreated  $\mu$  chain. We are therefore confident that the observed heavy chain size heterogeneity of cell surface immunoglobulin does not result from degradation of  $\mu$  chain during the isolation procedure.

A possible explanation for the heterogeneity of surface immunoglobulin is that there is a precursor-product relationship between the various species observed. Such a relationship could either reflect a biosynthetic pathway or metabolic turnover of membrane-associated immunoglobulin. In the first case the HL subunit would be an intermediate in the biosynthesis of  $H_2L_2$ , and the small heavy chain would be a partially glycosylated  $\mu$  chain requiring the addition of further sugar residues for completion. In the alternative situation, the direction would be expected to be towards degradation of  $H_2L_2$  to HL and proteolysis of the heavy chain. We have ruled out either possibility by culturing surface-labelled spleen cells *in vitro* for 0–12 h. The absence of any form of precursor-product relationship was clearly indicated by the fact that the ratios of  $H_2L_2$  to HL and large heavy chain (that is, a  $\mu$  chain) to small heavy chain determined at time zero remained the

same in cell surface and released immunoglobulin recovered at all times of incubation. This experiment demonstrated also that the half lives of all the immunoglobulin species present on the cell surface are similar, a value of 10 h being determined.

We then pursued the possibility that the small heavy chain was derived from a class other than IgM by using antisera of defined class specificity for isolation of surface immunoglobulin. Antibodies specific for  $\gamma 1$ ,  $\gamma 2a$  and  $\alpha$  chains did not precipitate immunoglobulin from detergent-solubilised, surface-labelled splenic lymphocytes. On the other hand, specific anti- $\mu$  chain-precipitated  $H_2L_2$  and HL containing heavy chain corresponding only in size to authentic, secreted  $\mu$  chain (Fig. 3*a*). This material may therefore be identified as  $\mu$  chain with certainty. When the supernatant from the anti- $\mu$  precipitation was further reacted with polyspecific anti-(mouse Ig), however, the  $H_2L_2$  and HL species precipitated were predominantly composed of the small heavy chain (Fig. 3*b*). We may therefore conclude that there is present on the surface of murine splenic lymphocytes a heavy chain larger than the  $\gamma$  chain, yet smaller than the  $\mu$  chain, which is not precipitated with antisera to  $\gamma$ ,  $\alpha$  or  $\mu$  chains, but which is reactive with a polyspecific antiserum to mouse Ig. As the small heavy chain was not precipitated by anti-( $\mu$  chain), the possibility that it is an incompletely glycosylated  $\mu$  chain<sup>14</sup> is ruled out.

The inescapable conclusion is that this heavy chain, which accounts for about 40% of heavy chain isolated from surface-labelled spleen cells, represents a hitherto undescribed class of



**Fig. 3** Two different heavy chain classes present on the surface of murine splenic lymphocytes. Spleen cells were labelled with  $^{125}\text{I}$  as described in the legend to Fig. 1. They were washed once in Eagle's medium containing 10% foetal calf serum, resuspended in the same medium at  $2 \times 10^7$  cells  $\text{ml}^{-1}$ , and then incubated at 37°C in an atmosphere of 5%  $\text{CO}_2$  in air for 6 h. At the end of the incubation period, the cells were 95% viable (trypan blue exclusion). The medium, containing released cell surface immunoglobulin, was separated from the cells by centrifugation (400g, 10 min), adjusted to contain 1% (w/v) Nonidet P40, and dialysed against 1% (w/v) Nonidet P40 in phosphate buffered saline. To the non-diffusate was added a specific rabbit anti-(mouse  $\mu$  chain) serum (10  $\mu\text{l}$ ) and goat anti-(rabbit IgG) serum (100  $\mu\text{l}$ ). The resulting precipitate was removed by centrifugation, and the supernatant was further reacted with polyspecific rabbit anti-(mouse Ig) (50  $\mu\text{l}$ ) followed by the appropriate amount of mouse Ig (40  $\mu\text{g}$ ) to ensure complete precipitation of mouse Ig. Both precipitates were washed and analysed in 4.2% (w/v) polyacrylamide gels as described in the legend to Fig. 1. The  $H_2L_2$  containing regions of both gels were eluted, reduced and alkylated, and analysed on 10% (w/v) polyacrylamide gels as described in the legend to Fig. 2. *a*, Reduced cell surface  $H_2L_2$  precipitated with rabbit anti-(mouse  $\mu$  chain); *b*, reduced cell surface  $H_2L_2$  prepared from the supernatant of the precipitation with rabbit anti-(mouse  $\mu$  chain) by addition of rabbit anti-(mouse Ig). —,  $^{125}\text{I}$  incorporated into cell-surface Ig; ---,  $^{131}\text{I}$ -labelled internal marker.

immunoglobulin in the mouse; the most obvious surmise is that it corresponds to the human IgD class. To support this suggestion we note the similar mobility on SDS-polyacrylamide gels of human  $\delta$  chain<sup>2</sup> and the novel heavy chain of mouse lymphocytes described above. This newly described mouse immunoglobulin, which we shall now refer to as IgD, is also similar to human IgD<sup>2</sup> in its marked susceptibility to proteolytic degradation (E.R.A. and R.M.E.P., unpublished). A similar conclusion has also been drawn by others (E. S. Vitetta and J. W. Uhr, unpublished).

Contrary to our expectation, however, was the finding of  $\mu$  chain, but not  $\delta$  chain, in foetal liver (16 d embryos) and neonatal spleen and liver. In addition, splenic lymphocytes of 6 week and 6 month old mice contained similar amounts of  $\delta$  chain, the ratio of  $\mu$ : $\delta$  being about 3:2. This could mean that the expression of IgD in the mouse is subsequent to the appearance of IgM. If this is not the case, then IgD-bearing lymphocytes must arise in organs other than spleen or foetal liver. Perhaps relevant to this point is the fact that IgD constitutes the major immunoglobulin class present on murine lymph node cells, where the  $\mu$ : $\delta$  ratio was found to be 1:3.5–4.0.

It is certainly intriguing that spleen and lymph node cells differ markedly in terms of the relative amounts of  $\mu$  and  $\delta$  chains present on their surfaces. In this respect we may note that spleen cells, but not lymph node cells, respond to lipopolysaccharide (G. Janosy and R.M.E.P., unpublished), and that spleen cells secrete largely IgM, whereas the major product of lymph node cells is IgG<sup>15</sup>. The presence or absence, however, of a functional relationship between these separate observations remains to be determined. Whether the molecular heterogeneity of total cell surface Ig is reflected in a similar pattern on individual cells is not known. Nonetheless, a relationship between this observed heterogeneity and regulation of the B-lymphocyte response to antigen is suggested.

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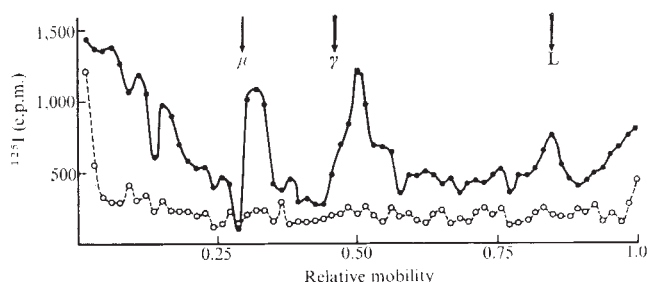
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## Immunoglobulin of T lymphoma cells is an integral membrane protein

IMMUNOGLOBULINS, predominantly 7S IgM molecules (molecular weight  $\sim 200,000$ ), are associated with the plasma membranes of lymphocytes<sup>1–3</sup> and considerable evidence suggests that these proteins function as receptors for antigen<sup>4–6</sup>. If these molecules are themselves integral parts of the lymphocyte membrane or are strongly associated with integral proteins they may transmit antigen-induced signals through the membrane and initiate cell activation. It has been proposed that the antigen-combining site of T cell IgM protrudes from the cell surface, but that the Fc portion bearing the characteristic antigenic determinants of the heavy chain is not accessible to large molecules in the solvent<sup>2,7</sup>. If these proposals are correct, then surface immunoglobulin of T cells might be considered an integral constituent of the plasma membrane<sup>8</sup>.

Cultured mouse WEHI-22 T lymphoma cells represent monoclonal T cells which synthesise and express surface IgM-like immunoglobulin<sup>2,5,9</sup> showing certain collaborative properties<sup>10</sup>. If immunoglobulin is an integral protein of WEHI-22 plasma membrane, it should be possible to isolate the plasma membrane and to detect immunoglobulin associated with it. Because preparation of purified membranes involves large dilution factors and prolonged washing<sup>11</sup>, it is likely that loosely associated peripheral proteins would be lost during the process. In this communication we provide evidence that immunoglobulin is detectable on purified plasma membrane of WEHI-22 cells and is therefore strongly associated with the T cell membrane.

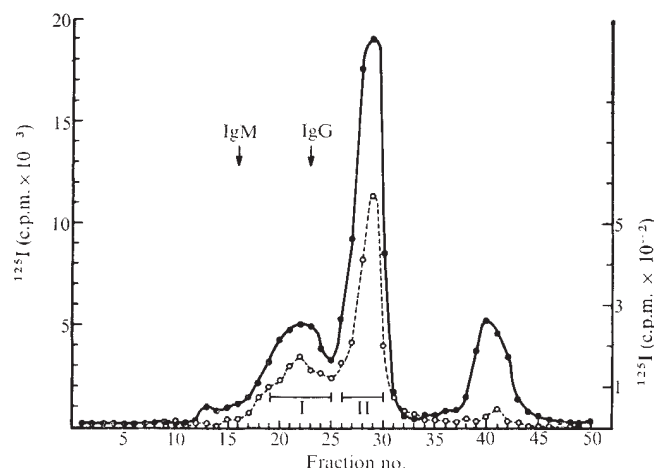
Plasma membranes were prepared from unlabelled WEHI-22 cells to which were added  $5 \times 10^7$  surface radioiodinated WEHI-22 cells. The cells were radioiodinated by a modification<sup>12</sup> of the lactoperoxidase method developed for labelling



**Fig. 1** Polyacrylamide gel electrophoresis in SDS of <sup>125</sup>I-labelled polypeptide chains of immunoglobulin isolated from membranes of T lymphoma cells of the line WEHI-22. Membrane protein (225  $\mu$ g) was solubilised in 500  $\mu$ l 1% Nonidet P40–6M urea. The solution was dialysed against PBS and subsequently the nondialysable protein was radioiodinated using chloramine-T (ref. 15): to 100  $\mu$ l membrane protein solution (0.45 mg protein ml<sup>-1</sup>) were added 10  $\mu$ l carrier-free <sup>125</sup>I (100 mCi ml<sup>-1</sup>) and 5  $\mu$ l chloramine-T (1 mg ml<sup>-1</sup>) and then the mixture was held at 4°C for 1 h. The reaction mixture was fractionated by gel filtration on Sephadex G100 in 0.5% Nonidet P40 in PBS. Fractions of the <sup>125</sup>I-labelled high molecular weight peak were pooled and PBS added to a total volume of 3.0 ml. Aliquots (200  $\mu$ l) of this <sup>125</sup>I-labelled membrane protein solution were precipitated with the specific precipitation system which consisted of mouse IgM (100  $\mu$ l of a concentration of 0.1 mg ml<sup>-1</sup>) and rabbit antiserum to mouse IgM (100  $\mu$ l of antiserum diluted 1 in 2) or with the control precipitation system which consisted of fowl IgG (100  $\mu$ l of a concentration of 0.1 mg ml<sup>-1</sup>) and rabbit antiserum to fowl IgG (100  $\mu$ l of antiserum diluted 1 in 2). The coprecipitates were washed three times with PBS, dissolved in a buffer containing 10% glycerol, 5% mercaptoethanol, 3% SDS in 0.125 M Tris-HCl, pH 6.8 which was 6M in urea and then analysed by polyacrylamide gel electrophoresis (10% acrylamide, 0.25% bisacrylamide) in a discontinuous buffer system according to the method of Laemmli<sup>16</sup>. ●, Specific precipitates; ○, control precipitates;  $\mu$ ,  $\gamma$  and L refer to positions of standard immunoglobulin chains.



lymphocytes<sup>13</sup>. The label was used solely to monitor the distribution of plasma membrane during fractionation and the subsequent analysis of the membrane proteins (Fig. 2). The cells were disrupted using a modification of the "pump" described by Wright *et al.*<sup>14</sup> and the plasma membrane was separated according to the method of Crumpton and Snary<sup>11</sup>. The purified membrane which exhibited a 35-fold enrichment in specific 5'-nucleotidase activity relative to the initial cell homogenate was solubilised in 1% Nonidet P40-6M urea. Approximately 95% of the radioactivity was obtained in solution. This solution was dialysed against PBS and the nondialysable protein radioiodinated using the chloramine-T method (ref. 15 and legend to Fig. 1). The reaction mixture was fractionated by gel filtration on Sephadex G-100. Approximately 70% of the added <sup>125</sup>I-iodide was associated with high molecular weight protein as judged by gel filtration.



**Fig. 2** Gel filtration on Sephadex 6B of solubilised membranes of T lymphoma cells of the line WEHI-22. Two procedures were used: *a*, membranes were solubilised in 1% sodium deoxycholate (Merck) in 20 mM Tris-HCl, pH 8.2 (0.5 mg membrane protein per ml solvent; 1 h at room temperature) and the Sephadex 6B column (55 cm × 0.8 cm) was developed in the same solvent (●). *b*, Membranes were solubilised in 10M urea-1.5 M acetic acid (0.5 ml membrane protein per ml solvent; 2 h at 30°C) followed by dialysis against Tris-NaCl, pH 8.0; 15% of the original membrane bound counts were soluble in Tris-NaCl as judged by centrifugation. Separation was performed on a Sephadex 6B column of identical dimensions, the elution buffer was PBS (○). The fractions indicated I and II obtained from the separation using procedure B were pooled, concentrated by ultrafiltration and radioiodinated using chloramine-T<sup>15</sup>. These fractions were assessed for the presence of Ig by coprecipitation (legend to Fig. 1). IgM and IgG represent the position of intact marker proteins (molecular weights 900,000 and 150,000, respectively) resolved under these conditions.

The presence of Ig in the solubilised <sup>125</sup>I-labelled membrane was ascertained by immunological precipitation. We found that the specific immune precipitate possessed  $7.7 \pm 0.5 \times 10^3$  c.p.m. whereas the control precipitate gave  $1.1 \pm 0.1 \times 10^3$  c.p.m. This difference shows that the plasma membrane contains Ig. This interpretation is supported by polyacrylamide gel electrophoretic analysis of the immune precipitates in buffers containing sodium dodecylsulphate (SDS)<sup>16</sup>. Furthermore, the results of these analyses closely resembled those reported elsewhere for WEHI-22 surface Ig isolated from lactoperoxidase-iodinated whole cells (ref. 2 and D.H., J.J.M. and A. W. Harris, submitted for publication). Radioiodinated plasma membrane Ig contained components resembling  $\mu$ -chain and L-chain in their electrophoretic mobilities (Fig. 1). A third component was also present with a mobility consistent with a molecular weight of about 40,000. Studies to be reported in detail elsewhere revealed that this component has properties similar to those predicted for the surface membrane receptor for the Fc-portion of exogenous IgG (ref. 17). A considerable amount of material with mobilities greater than that corresponding to a molecular weight of  $10^5$  was observed in all

polyacrylamide gels of homologous immune precipitates derived from preparations of plasma membrane radioiodinated using chloramine-T. The amount and distribution of this material was, however, variable and studies of its nature were not pursued further; similar material was observed in Ig isolated from murine B cells radioiodinated using chloramine-T (E. White, and J.J.M., unpublished observations).

These results establish that light chains and  $\mu$ -like heavy chains were present in surface membrane Ig of WEHI-22 cells; but, they do not provide any information regarding the intact state of the molecule. This problem has been approached by fractionating solubilised membrane by gel filtration on Sepharose 6B (legend to Fig. 1) and radioiodinating the fractions eluted in positions corresponding to molecular weights of  $<10^5$  and between  $1 \times 10^5$  and  $3 \times 10^5$  in order to ascertain the presence or absence of free polypeptide chains and 7S Ig respectively. These fractions are illustrated in Fig. 2. The fractions termed I and II were pooled as indicated and radioiodinated using chloramine-T (ref. 15). Immunoglobulin estimated by immune precipitation, was found only in the 100,000-300,000 molecular weight fraction ( $3.6 \pm 0.6 \times 10^3$  c.p.m. specific;  $1.1 \pm 0.1 \times 10^3$  c.p.m. control). No Ig was detected in the lower molecular weight fraction ( $0.9 \pm 0.2 \times 10^3$  c.p.m. specific;  $1.1 \pm 0.2 \times 10^3$  c.p.m. control). There was insufficient material in the elution position of 19S IgM to enable analysis of this fraction. These results are consistent with the previous observation that the Ig associated with the surface of T cells exists primarily as 7S units<sup>2,5</sup> rather than as free polypeptide chains<sup>18</sup>.

Surface Ig of mouse T lymphoma cells of the line WEHI-22 was found to exist as a 7S IgM unit which was bound to the plasma membrane sufficiently strongly to resist dissociation or elution during the disruption of the cells and the isolation of the plasma membrane. These results are consistent with the proposals that either T cell immunoglobulin is itself an integral membrane protein or that it is tightly associated with another component buried in the lipid bilayer of the membrane. Similar proposals also apply to the surface IgM of the human lymphoblastoid cell line BR18 (M. J. Hayman, and M.J.C., unpublished observations).

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## Antibody activity associated with immunoglobulins synthesised by human lymphoblastoid cell lines

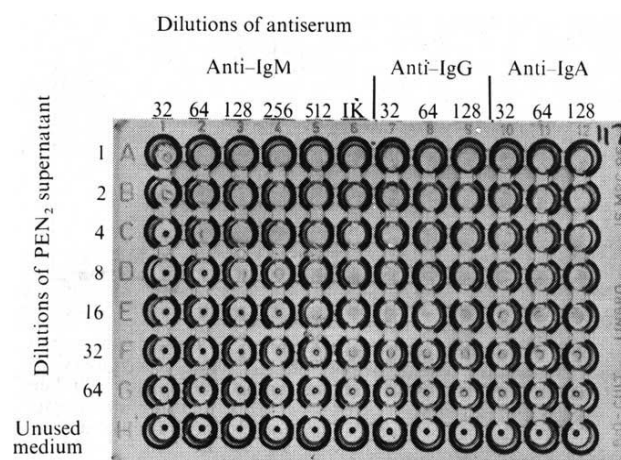
ANTIBODY activity has not previously been detected in association with immunoglobulins synthesised spontaneously by human lymphoblastoid cell lines *in vitro*. One reviewer has commented "if some antibody activity was to be found, the established cell lines would be a fascinating tool for the study of homogenous antibodies"<sup>1</sup>. We here report such a finding.

In the course of studies on immunoglobulin (Ig) production *in vitro*, by means of a haemagglutination-inhibition assay<sup>2</sup>, supernatants from four human lymphoblastoid lines (of more than 60 tested) were found to agglutinate glutaraldehyde-fixed Ig-coated sheep erythrocytes in the absence of anti-Ig serum. Details of the four lines are given in Table 1. It was established that the Ig coating is irrelevant to the agglutination reaction and that supernatants from these four lines (but not from others) will agglutinate sheep or human erythrocytes fixed with glutaraldehyde or formaldehyde. There is no detectable activity against fresh erythrocytes of either species, nor against glutaraldehyde or formalin-fixed calf erythrocytes. Tanned or glutaraldehyde-fixed horse erythrocytes agglutinate spontaneously in the presence of unused culture medium (Ham's F10 with 10% tryptose phosphate broth and 20% foetal calf serum). The agglutinating activity against glutaraldehyde-fixed sheep red cells seems to be independent of temperature over the range 4° C to 37° C.

The kinetics of agglutinin production have been studied in cell line PEN<sub>2</sub>. Some activity is detectable within 24 h of resuspending the cells at 10<sup>5</sup> ml<sup>-1</sup> in fresh medium. The titre

then rises steadily to reach a plateau by about day 5 although the cells continue in log phase growth for a further 5–6 d.

Inhibition of agglutinating activity was attempted with monospecific antisera directed against each of the major classes of human Ig heavy and light chains, in experiments of the type shown in Fig. 1. The results with three of the lines were clear cut (Table 1), indicating that the activity is associated with Ig of class IgM $\lambda$  in the supernatants of HIL<sub>1</sub>, ANA<sub>1</sub> and PEN<sub>2</sub>. In the case of BRI<sub>8</sub> the agglutinating titre of the supernatant is low and the findings less easy to interpret. Anti- $\kappa$  serum consistently inhibits agglutination, but both anti-A and anti-M (and occasionally anti-G) also seem to have some inhibitory effect. Attempts to identify Igs by immunoelectrophoresis of concentrated supernatants from cell lines ANA<sub>1</sub>, PEN<sub>2</sub> and BRI<sub>8</sub> were only partially successful. The presence of  $\lambda$  light chains in material from ANA<sub>1</sub> and PEN<sub>2</sub> was confirmed but no other heavy or light chains could be demonstrated. Failure to detect Igs, when their initial concentration in culture supernatant is low, is common with this technique<sup>2</sup>.



**Fig. 1** Haemagglutination carried out in round-bottomed wells of microtest II plate (Linbro plastics). Reciprocal dilutions of PEN<sub>2</sub> supernatant (in fresh culture medium containing 20% foetal calf serum) are recorded on the left; reciprocal dilutions of monospecific anti-human Ig chain sera (in physiological saline) are recorded above. One drop of diluted supernatant was mixed with 1 drop of diluted antiserum in each well and 30 min later 1 drop of a 1% suspension (in saline) of sheep red cells, previously fixed in 2.5% glutaraldehyde, was added. The cells were allowed to settle overnight at room temperature. Agglutination is shown by the absence of a dark central pellet of erythrocytes. Inhibition of the agglutination reaction becomes more marked with increasing concentrations of anti-IgM serum. There is no inhibition demonstrable with anti-IgG or anti-IgA sera.

**Table 1** Cell lines producing agglutinin against fixed sheep or human erythrocytes

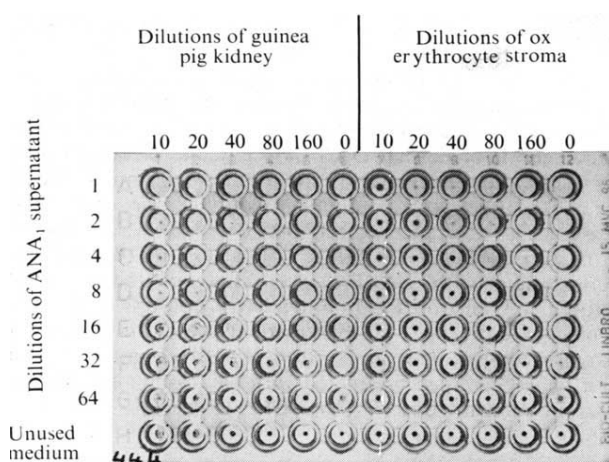
| Donor            |     |                                 | Shell cell agglutinin in supernatant |                |        |        |               |   |   |        |        |        |     |       |
|------------------|-----|---------------------------------|--------------------------------------|----------------|--------|--------|---------------|---|---|--------|--------|--------|-----|-------|
| Cell line        | Sex | Diagnosis                       | Laboratory of origin*                | Maximum titre† | Anti-G | Anti-A | Inhibited by‡ |   |   | Anti-M | Anti-K | Anti-L | GPK | OxRBC |
| ANA <sub>1</sub> | M   | Healthy infant                  | MRC                                  | 1 : 32         | —      | —      | +             | — | + | —      | —      | —      | —   | +     |
| HIL <sub>1</sub> | F   | Healthy infant                  | MRC                                  | 1 : 32         | —      | —      | +             | — | + | —      | —      | —      | —   | +     |
| PEN <sub>2</sub> | M   | Klinefelter's syndrome (47,XXY) | MRC                                  | 1 : 32         | —      | —      | +             | — | + | —      | —      | —      | —   | —     |
| BRI <sub>8</sub> | M   | Healthy adult                   | Searle                               | 1 : 8          | ±      | +      | +             | + | + | —      | —      | —      | —   | —     |

\* MRC is the authors' laboratory; Searle, G. D. Searle and Company, Research Division, High Wycombe, UK.

† Maximum dilution of supernatant giving positive result against 1% suspension of glutaraldehyde-fixed sheep erythrocytes. Titres against human cells or against formalin-fixed sheep erythrocytes are usually lower. Recently, using an 0.25% suspension of glutaraldehyde-fixed sheep erythrocytes, titres of up to 1 : 256 have been observed in PEN<sub>2</sub> supernatants.

‡ Anti-G, Anti-A and Anti-M, Monospecific goat anti-human heavy chain sera (Technicon); Anti-K and Anti-L, monospecific rabbit anti-human light chain sera (Hoechst); GPK, OxRBC, guinea pig kidney and ox erythrocyte stroma extracts for infectious mononucleosis ('Monospot') tests (Ortho diagnostics).





**Fig. 2** Haemagglutination test set up as in Fig. 1. Reciprocal dilutions of ANA<sub>1</sub> supernatant are recorded on the left. One drop of the diluted supernatant was mixed with one drop of guinea pig kidney or ox erythrocyte stroma extracts (Ortho Diagnostics) in saline (reciprocal dilutions shown above) in each well of the test plate. One drop of glutaraldehyde-fixed sheep erythrocytes (1% suspension in saline) was added 20 min later and the plate left overnight at room temperature. Progressive inhibition of agglutination with increasing concentrations of ox erythrocyte stroma is clearly demonstrated. Guinea pig kidney extract has no apparent effect on the agglutination reaction.

Preliminary studies on the physical properties of the erythrocyte-agglutinating factor from PEN<sub>2</sub> supernatants show that it is precipitated by 10–40% saturated ammonium sulphate. It can be isolated as a single sharp peak from a Sephadex G200 column and the major fraction bands with a human IgM marker on a 10–40% sucrose gradient. There is a minor peak of agglutinating activity in the lower molecular weight range, possibly representing depolymerised IgM.

There seems to be no common factor in the lines or in their origins which distinguishes these four cultures from others established and maintained in identical conditions. The four lines are readily distinguished from each other by cytogenetic and isoenzyme analysis. A second line, ANA<sub>2</sub>, was established from the same blood sample which gave rise to ANA<sub>1</sub>. No erythrocyte agglutinating activity is detectable in its supernatant.

The original sera from the donors of PEN<sub>2</sub>, ANA<sub>1</sub> and HIL<sub>1</sub> were recovered from storage at –20° C along with samples from 20 other donors including three cord bloods and nine from patients with Klinefelter's Syndrome (karyotype 47,XXY or 49,XXXXY). Lymphoblastoid cell lines had been established from most of these control donors. The sera were tested for agglutinating activity against glutaraldehyde-fixed sheep red cells. More than half (including those from the donors of PEN<sub>2</sub> and HIL<sub>1</sub>) gave weak reactions at dilutions of 1 : 5 or 1 : 10. These were considered non-specific. Four Paul-Bunnell positive samples from patients with infectious mononucleosis agglutinated the fixed sheep cells at titres of 1 : 160 to > 1 : 640. As in the Paul-Bunnell reaction, this agglutination was inhibited by adsorption with ox erythrocyte stroma but not with guinea pig kidney extract<sup>3</sup>. Similar tests on the four cell line supernatants show that those from ANA<sub>1</sub> and HIL<sub>1</sub> consistently behave like infectious mononucleosis sera (Fig. 2), but agglutinating activity in PEN<sub>2</sub> or BRI<sub>3</sub> supernatants seems not to be inhibited by ox erythrocyte or by guinea pig kidney extracts.

These preliminary data suggest that EB virus, which is implicated both in infectious mononucleosis<sup>4</sup> and in the proliferation of human lymphoblastoid cell lines *in vitro*<sup>5,6</sup>, may be responsible for the production of agglutinins directed against fixed sheep erythrocytes by at least some of these lines.

Further work is in progress to isolate and compare the agglutinins synthesised by the four cell lines under study.

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## Tolerance induction with bovine $\gamma$ globulin in mouse radiation chimaeras depends on macrophages

THE injection of adult mice with ultracentrifuged heterologous  $\gamma$  globulin induces immunological unresponsiveness to a subsequent injection of the globulin incorporated in incomplete or complete Freund's adjuvant<sup>1</sup>. It has been reported that inbred strains of mice differ in their susceptibility to the induction of unresponsiveness. Bovine  $\gamma$  globulin (BGG), human  $\gamma$  globulin (HGG) and rabbit  $\gamma$  globulin (RGG) easily produce tolerance in C57BL/6 and DBA/2 mice but not in BALB/c mice<sup>2,3</sup>. Passage of BGG through the circulation of BALB/c mice (but not DBA/2 mice) gives preparations which produce a high degree of tolerance in the recipient mice suggesting that strain differences in the induction of tolerance are related to the capacity of the reticuloendothelial system to process the immunogenic component in ultracentrifuged soluble BGG (sBGG). Here, we report that the administration of carrageenin, which is known to be toxic for macrophages destroys the resistance to tolerance induction in BALB/c mice. Also spleen and bone marrow cells transferred from BALB/c mice to irradiated DBA/2 mice to form chimaeras, do not show characteristic BALB/c resistance to induction of tolerance until twelve weeks after transfer.

To test immune responsiveness to BGG we used previously described procedures<sup>2,3</sup>. One week after intraperitoneal (i.p.) injection of 2.0 mg sBGG for tolerance induction the mice

**Table 1** Effect of various doses of carrageenin on the induction of unresponsiveness with 2 mg in BALB/c mice

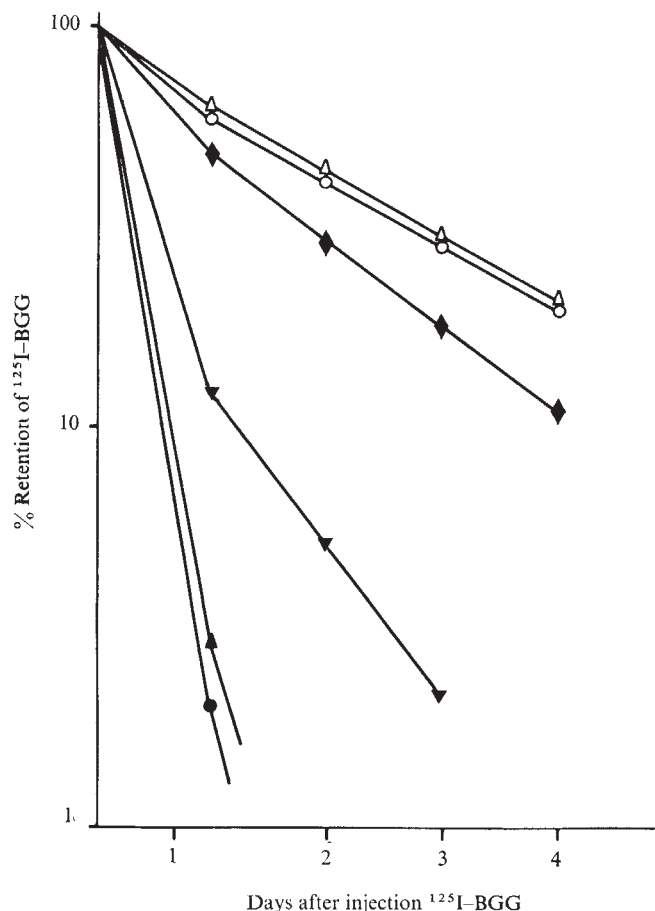
| Carrageenin treatment                     | Immune responsiveness |                    |        |
|-------------------------------------------|-----------------------|--------------------|--------|
|                                           | Tolerant              | Partially tolerant | Immune |
| 0.5 mg 3 d and 1 d before sBGG            | 2                     | 3                  | 4      |
| 4 × 0.5 mg every second day before sBGG   | 11                    | 6                  | 2      |
| 4 × 0.5 mg every second day before saline |                       |                    | 5      |
| None                                      |                       |                    | 5      |

Kappa carrageenin (Marine Colloids) was dissolved in saline by heating, and an appropriate dose administered in 0.5 ml. 2.0 mg sBGG was administered 24 h after the last injection of carrageenin. One week later the mice were immunised with 100  $\mu$ g BGG-CFA and then challenged with 2  $\mu$ g <sup>125</sup>I-BGG.

were immunised with 100 µg BGG in complete Freund's adjuvant (CFA). Five days later they were given 0.1% KI in their drinking water. One week after immunisation the mice were challenged with 2 µg  $^{125}\text{I}$ -BGG and whole body counts obtained daily in a gamma scintillation counter. Two milligrams sBGG readily induces tolerance in DBA/2 mice. BALB/c mice exhibit complete resistance to the induction of unresponsiveness at this dose and show subsequent clearance rates of  $^{125}\text{I}$ -BGG indistinguishable from the immune control animals.

Carrageenin, a polysaccharide isolated from seaweed, known to be toxic for macrophages<sup>4</sup> but not for lymphocytes<sup>5,6</sup> was tested for its effect on tolerance induction by administration to BALB/c mice, before the injection of sBGG. Pretreatment with carrageenin favours tolerance induction in BALB/c mice (groups 1 and 2) whereas the immune response of the mice treated with BGG-CFA only (group 3) was not affected (Table 1). Thus, carrageenin-sensitive cells seem to be a critical factor in susceptibility to the induction of unresponsiveness.

We studied further this difference using cell transfer experiments. DBA/2 mice share the major histocompatibility loci (*H-2<sup>d</sup>*) with BALB/c mice and are poor responders to most bone marrow allografts<sup>7,8</sup>. Previous spleen cell transfer experiments showed that although the animals were true chimaeras with respect to their content of donor T and B cells at 4 weeks after lethal irradiation and reconstitution, they responded to the induction of tolerance to sBGG like normal animals of recipient strains<sup>9</sup>. We wished to examine the change with time of the susceptibility to the induction of unresponsiveness in lethally irradiated (850 rad) DBA/2 mice reconstituted with  $2 \times 10^7$  BALB/c bone marrow cells from 8-week-old donors.



**Fig. 1** Induction of tolerance in DBA/2 mice 12 weeks after the reconstitution with BALB/c bone marrow cells. Clearance rate 2 µg  $^{125}\text{I}$ -BGG in normal (N) and irradiated-reconstituted (I-R) animals:  $\Delta$ , N, saline-CFA;  $\circ$ , I-R, saline-CFA;  $\blacklozenge$ , N, 2 mg sBGG + BGG-CFA;  $\blacktriangledown$ , I-R, 2 mg sBGG + BGG-CFA;  $\blacktriangle$ , N, BGG-CFA;  $\bullet$ , I-R, BGG-CFA.

This selected host-donor combination displayed practically no secondary disease and low mortality (5 out of 45 animals, in 4 months after irradiation).

As in previous spleen cell transfer experiments, animals tested for tolerance induction 4 weeks after reconstitution with BALB/c bone marrow cells showed non-immune clearance characteristic of normal DBA/2 mice made tolerant (Table 2). Six weeks after reconstitution the results were similar. But after 12 weeks 10 out of 11 animals exhibited the characteristic resistance to tolerance of the BALB/c donors. At that time 2 mg sBGG failed to induce tolerance in DBA/2 animals reconstituted with BALB/c cells although the same dose induced tolerance in the non-irradiated controls (Fig. 1). No significant difference was observed in the ability of BALB/c marrow-reconstituted and normal DBA/2 mice to clear 2 µg  $^{125}\text{I}$ -BGG when pretreated with CFA only, or to respond to an immunising dose of BGG-CFA (Fig. 1).

**Table 2** Induction of unresponsiveness to sBGG in irradiated DBA/2 mice reconstituted with BALB/c bone marrow cells

| Immune responsiveness | Weeks after reconstitution |   |    |
|-----------------------|----------------------------|---|----|
|                       | 4                          | 6 | 12 |
| Tolerant              | 8                          | 5 | 0  |
| Partially tolerant    | 0                          | 1 | 1  |
| Immune                | 0                          | 0 | 10 |

Mice are irradiated with 850 rad and reconstituted with  $2 \times 10^7$  bone marrow cells. They were injected with 2 mg sBGG 4, 6, or 12 weeks after reconstitution. One week later the mice were immunised with 100 µg BGG-CFA and then challenged with 2 µg  $^{125}\text{I}$ -BGG.

These results demonstrate that the resistance of BALB/c mice to tolerance induction resides in a cell population sensitive to the action of carrageenin. The most likely candidate would be macrophages in the reticuloendothelial system which are responsible for effectively processing the immunogenic portion of BGG and initiating a response in some clones before tolerance is induced in the remainder. On subsequent immunisation these primed cells proceed to make antibody and the mice seem on balance to be immune. The literature is full of observations demonstrating an enhanced immune response following stimulation of macrophages and suppression following their destruction<sup>10</sup>.

What is somewhat surprising is the failure of spleen and bone marrow cell grafts to effect reconstitution of an irradiated recipient in the sense that they would then behave like donors in respect to tolerance induction. Balner<sup>11</sup> however, has shown that after irradiation and bone marrow grafting, host type macrophages in the peritoneum were not replaced to any major extent for a significant time. The behaviour of our irradiation chimaeras agrees with this observation. Four weeks after grafting, animals possessed T and B cells of donor origin but continued to behave as normal hosts with respect to ease of tolerance induction<sup>9</sup>. We have demonstrated that even 6 weeks is insufficient for functional reconstitution but after 12 weeks (Table 1) the behaviour of the chimaeras was now almost totally like the donor as to tolerance induction.

These results provide strong confirmation of the existence of a decision making event at the level of the macrophage that determines whether in a particular strain of mice the injection of an antigen such as BGG will result in tolerance or immunity.

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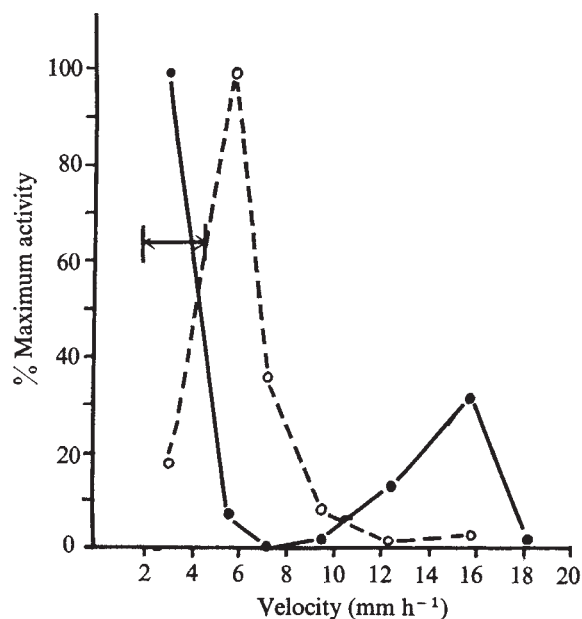
## Separation of T effector cells in humoral and cellular immunity

THE precise relationship between thymus-derived (T) helper cells in the antibody response and T effector cells in cellular immunity is not clear. Both cell functions have closely related dose response curves<sup>1</sup>, kinetics of sensitisation<sup>1</sup>, susceptibility to antibody suppression<sup>1–3</sup> and anatomical distribution<sup>4</sup>. Bone marrow-derived (B) lymphocytes can be stimulated for antibody synthesis by a stimulus from immunocompetent allogeneic<sup>5</sup> or xenogeneic<sup>6</sup> lymphoid cells. These observations have led to the hypothesis that T helper cells are identical to cytotoxic T cells and act on B cells by secreting a factor which stimulates them to produce antibody. Liew and Parish<sup>7</sup> have reported, however, that T helper cells in humoral immunity recognise different forms of chemically modified flagellin than do T cells in cellular immunity. Furthermore, Dennert<sup>8</sup> has shown that T-helper and T-killer cells are mutually exclusive under certain immunisation conditions. The reports of Dennert<sup>8</sup> and Liew and Parish<sup>7</sup> suggest that T helper cells may not be identical to T effector cells in cellular immunity. We have now used a rosette technique<sup>9</sup> which distinguishes different degrees of antigen-binding, in combination with velocity sedimentation at unit gravity<sup>10</sup> to distinguish and separate T cells involved in the helper effect from T cells participating in cellular immunity reactions.

We have shown (work in preparation) that sheep red blood cells (SRBC) in complete Freund's adjuvant (CFA) injected into C57BL/6 mice (Canadian Breeding Laboratories) induce both humoral and cellular immune reactions within 9 d after immunisation. For both of these responses T cells are necessary: T-helper cells trigger B cells for antibody synthesis<sup>11</sup>; T cells in cellular immunity are involved in delayed hypersensitivity (DHR) and *in vitro* cytotoxicity (our work in preparation). This system was therefore useful for investigating the relationship between T cells in humoral and cellular immunity in the same experimental animals and within the same lymphoid organ.

DHR and helper cell activity were assayed by adoptive transfer systems as reported previously (our work in preparation). To detect DHR activity, immune cells were injected with  $5 \times 10^7$  SRBC intradermally into one footpad of normal animals. The other footpad received SRBC alone. The percentage in footpad volume after challenge was proportional to the number of immune cells injected. The DHR activity of SRBC immune cells was antigen-specific, sensitive to anti- $\theta$  serum plus complement, but insensitive to anti- $\theta$  serum alone (our work in preparation).

To assay for helper cell activity, immune cells and  $2 \times 10^8$  SRBC were injected with or without  $2 \times 10^7$  bone marrow cells into lethally irradiated recipients and the number of 19S plaque-forming cells (PFC) per spleen was determined 8 d later. An increase in the number of splenic PFC when an immune cell population was injected with bone marrow cells indicated helper cell activity in that population.



**Fig. 1** Sedimentation velocity profiles of 19S helper cells and DHR effector cells from immune lymph nodes. C57BL/6 mice received an intradermal injection of  $5 \times 10^7$  SRBC in CFA in the footpad. After 9 d, lymph node cells were removed, and subjected to rosette formation and sedimented with azide present. To assay for DHR, each fraction was injected with SRBC into the footpad of normal animals and the percentage increase in footpad volume was determined after 18 h. Footpad volume was measured by the deflection observed when the mouse foot was immersed in mercury on a Mettler 1600 balance to a mark made immediately distal to the lateral malleolus as described previously (our work in preparation). Three readings were made on each footpad and averaged. The difference in readings before and after skin testing was proportional to the increase in footpad volume. Percentage increase in footpad volume was calculated by dividing the difference by the original reading and multiplying by 100%. The relative activity was determined from a dose-response curve obtained with unfractionated immune cell (our work in preparation). To assay for helper cell activity, each fraction with  $2 \times 10^8$  SRBC was injected either alone or with  $2 \times 10^7$  normal bone marrow cells into syngeneic irradiated (750 r.) mice. 19S PFC per spleen were assayed 8 d later<sup>16</sup>. Helper cell activity was calculated by subtracting the number of splenic PFC in mice injected with that fraction alone from that of the fraction plus bone marrow cells. ○, 19S helper cell activity; ●, DHR activity. The velocity range of small lymphocytes (↔) has been indicated for convenience<sup>14</sup>.

19S helper cells sedimented as medium lymphocytes at 5–7 mm h<sup>-1</sup> (Fig. 1). DHR effector cells sedimented over a wider range of velocities (3–7 mm h<sup>-1</sup>), indicating the presence of both small and medium lymphocytes. When immune cells were subjected to rosette formation before sedimenting in the presence of sodium azide (to stabilise T rosettes<sup>9,13</sup>), rosettes sedimented at velocities greater than 6.5 mm h<sup>-1</sup>, as reported<sup>13</sup>. Rosette formation, however, had no effect on the sedimentation velocity of 19S helper cells (Fig. 1). DHR activity remained in the non-rosette forming small lymphocyte region (3 mm h<sup>-1</sup>) (Fig. 1). Activity in the medium lymphocyte region, however, shifted to the higher velocity region (13–15 mm h<sup>-1</sup>), indicating that medium lymphocyte effector cells had formed rosettes. Without azide in the sedimentation medium T rosettes dissociated and medium lymphocyte effector cells sedimented as single cells (5–7 mm h<sup>-1</sup>). The calculated number of SRBC (bound to make a medium lymphocyte RFC sediment at 13–15 mm h<sup>-1</sup>) is 8–10 SRBC (our work in preparation). Those antigen-binding properties are characteristic of T-type RFC (ref. 9).

These results indicate that 19S helper cells in immune animals are distinct from DHR effector cells. The latter were of two types: (a) non-rosette forming small lymphocytes and (b) medium lymphocyte T-type RFC. We have also found that SRBC-specific effector cells in *in vitro* cytotoxicity against sheep fibroblasts have the same size, antigen-binding properties, density

and sensitivity to anti- $\theta$  serum plus complement as DHR effector cells. These findings indicate that under the conditions of rosette formation used in the present study, 19S helper cells were distinct from T effector cells in cellular immunity.

Since the helper cell assay takes 8 d while the DHR and *in vitro* cytotoxicity assays take only 24 h, maturation of T helper precursor cells to T helper cells could occur. These experiments, however, strongly suggest that helper cells (or precursors) and T effector cells in cellular immunity are two different cells.

Recently, subsets of T cells with specific functions have been described. Cantor and Asofsky<sup>14</sup> have shown that two T cells with different anatomical distribution and sensitive to anti-lymphocyte serum synergise in the graft-versus-host reaction. Bach *et al.*<sup>15</sup> demonstrated that T cells involved in the mixed lymphocyte reaction are distinct from cytotoxic T cells. The distinction between 19S T-helper cells and T-effector cells in cellular immunity in the present study suggests a further division in T-cell subsets which is characterised in part by different abilities to bind antigen.

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Leukocyte migration inhibition was performed by employing 20  $\mu$ l capillary tubes (Drummond Scientific Co., USA) as described earlier<sup>6</sup>. Peripheral leukocytes from each individual were washed four times in Hanks' balanced salt solution and then adjusted to  $3 \times 10^{-7}$  cells ml<sup>-1</sup> in Eagle's basal medium (Grand Island Biological, USA) with 10% heat-inactivated foetal bovine serum (BioCult, UK). The capillaries were filled with the cell suspension, sealed at one end and centrifuged at 950g for 5 min. These were cut at the cell-medium interface and immersed in the medium to which had been added an EBV-antigen preparation from P3HR-1 cell lysate<sup>6</sup>. The final concentration of the EBV-antigen preparation in the medium was 1:5. A minimum of eight replicate cultures was set up for each sample. Similarly, control cultures were set up in medium without EBV-antigen preparation. The cultures were incubated at 37°C in a humidified atmosphere of 5% carbon dioxide for 16 h.

The extent of leukocyte migration was determined by multiplying the greatest diameter of the area of cell migration by its perpendicular diameter measured by an ocular micrometer and the percentage inhibition was calculated as:

$$\left[ 1 - \frac{(\text{mean extent of migration with antigen})}{(\text{mean extent of migration without antigen})} \right] \times 100$$

From the distribution of results (Table 1), we have considered a response positive when the percentage inhibition is 20% or greater, negative if smaller than 15% and equivocal if in between.

To test whether the effects of the EBV-antigen preparation on leukocyte migration could be abrogated by serum, sera from normal individuals (which had been stored at -20°C for up to 8 weeks) and from subjects (cases 1, 2 and 3) at the acute and the convalescent phase of IM (fresh sera) were used. One ml of serum at various concentrations was mixed with an equal amount of the EBV-antigen preparation. The mixture was incubated at 37°C for 30 min. Leukocyte migration inhibition was carried out as described, using as test cells peripheral leukocytes (from a normal adult, case 7) known to have their migration inhibited by the EBV-antigen preparation in the presence of foetal bovine serum alone. The final concentration of EBV preparation in the serum-antigen mixture used in the medium was 1:5. EBV-antigen preparation and serum put up separately at the same concentration in the medium were used as controls. The results of these experiments are listed in Table 2.

In addition, the acute sera (stored at -20°C for 14 d) and the freshly collected convalescent sera from cases 1, 2 and 3 were used to abrogate the effect of EBV-antigen preparation on leukocyte migration using autologous and heterologous leukocytes collected at the convalescent phase of IM. The results of these experiments showed that acute and convalescent sera from all three cases blocked the EBV-induced migration inhibition of autologous and heterologous leukocytes. In addition, the blocking effect observed decreased with increasing dilution of serum. Leukocytes and sera from cases 4 and 5 (Table 1) were used in a similar experiment, in which the antigen specificity of the blocking effect was indicated, using *Escherichia coli* as the irrelevant antigen (Table 3).

Acute sera from cases 1 and 4 were fractionated in a Sephadex G-200 column (2.5  $\times$  100 cm, Pharmacia, Uppsala) at a flow rate of 20 mlh<sup>-1</sup>. The IgG and IgM fractions were collected and examined at 28 nm in a Beckman spectrophotometer.

Immunodiffusion of these fractions against standard rabbit anti-human IgG and anti-human IgM (Behringwerke,) in 0.8% agarose (Seakem Agarose, Marine Colloids Inc., USA) buffered at pH 7.6 showed that the IgG fraction reacted with the anti-human IgG but not with anti-human IgM standard serum. Similarly, the IgM fraction reacted with anti-human IgM standard serum only. Immunofluorescent testing showed that both fractions contained antibodies to the capsid antigen of EBV. Blocking activity to the EBV antigen preparation was carried out as described and was detected only in the IgG fraction.

## Cell-mediated immunity to Epstein-Barr virus and a blocking factor in patients with infectious mononucleosis

ALTHOUGH the humoral immune response to Epstein-Barr virus (EBV) in infectious mononucleosis (IM) has been studied extensively<sup>1,2</sup>, little is known about cell-mediated immunity (CMI) to EBV in the disease. We describe here the status of CMI to EBV, and the presence of a blocking factor which can abrogate leukocyte migration inhibition, in patients with IM.

Serum antibody to EBV-capsid antigen in normal adults and patients with IM were measured by indirect immunofluorescence<sup>3</sup>, using SH-RP cells<sup>4</sup> previously irradiated with ultraviolet light to enhance virus capsid antigen<sup>5</sup>. The results are shown in Tables 1 and 2.



**Table 1** Serum antibody to EBV-capsid antigen and CMI to EBV detectable by leukocyte migration inhibition

| Case no. | Health status    | Serum-antibody titres to EBV-capsid antigen* | Paul-Bunnell titre | CMI to EBV as detected by leukocyte migration inhibition† |             |
|----------|------------------|----------------------------------------------|--------------------|-----------------------------------------------------------|-------------|
| 1        | IM acute         | 320                                          | 896                | — (−2.3±3.1)                                              |             |
|          | convalescent‡    | 320                                          | —                  | +                                                         | (72.1±2.5)  |
| 2        | IM acute         | 80                                           | 55                 | ±                                                         | (15.8±3.8)  |
|          | convalescent‡    | 160                                          | +                  | +                                                         | (27.0±1.3)  |
| 3        | IM acute         | 80                                           | 112                | —                                                         | (−11.9±1.7) |
|          | convalescent‡    | 40                                           | —                  | —                                                         | (26.0±1.2)  |
| 4        | IM acute§        | 160                                          | +                  | —                                                         | (2.0±3.1)   |
| 5        | IM convalescent§ | 40                                           | —                  | +                                                         | (20.0±2.5)  |
| 6        | Normal           | 20                                           |                    | +                                                         | (31.0±4.3)  |
| 7        | Normal           | 160                                          |                    | +                                                         | (24.0±2.1)  |
| 8        | Normal           | 40                                           |                    | +                                                         | (35.0±2.6)  |
| 9        | Normal           | 80                                           |                    | +                                                         | (42.5±2.5)  |
| 10       | Normal           | 320                                          |                    | +                                                         | (34.0±1.3)  |
| 11       | Normal neonate   | 0                                            |                    | —                                                         | (5.0±3.0)   |

\*Assayed by immunofluorescence<sup>3</sup> using SH-RP cells<sup>4,5</sup>.

†Mean % inhibition ± s.e. as determined from a minimum of eight replicate cultures. 20% or greater is marked as +, less than 15% as —, and in between as ±.

‡Collected 10–14 d after collection of acute sera, at convalescent phase of the illness; no Paul-Bunnell heterophil antibodies present.

§Tests performed in 10% equine rather than foetal bovine serum.

||Mono-spot test.

CMI is important in defence against infection with most viruses, including oncogenic viruses<sup>8,9</sup>. As well as causing IM, EBV is a potential oncogenic virus in man because of its ability to transform human haematopoietic cells *in vitro*<sup>4,10</sup>, its ability to cause lymphomas in non-human primates<sup>11,12</sup> and its association with at least two types of human tumour, Burkitt's lymphoma<sup>13</sup>, and nasopharyngeal carcinoma<sup>14</sup>.

We have shown that CMI to EBV was not detected *in vitro* in circulating leukocytes in the acute phase of IM (Table 1), but was found in circulating leukocytes of normal adults who had antibody to EBV and patients convalescent from IM. In

**Table 2** Blocking effect by sera from patients with IM on leukocyte migration inhibition and its absence in normal sera

| Case no.      | Serum anti-EBV capsid antibody titre | % Inhibition* |                 |
|---------------|--------------------------------------|---------------|-----------------|
|               |                                      | Serum alone   | Serum + EBV     |
| 1 acute†      | 320                                  | — (−3.2±2.7)  | —   (−4.8±2.4)  |
| convalescent‡ | 320                                  | — ( 5.2±1.3)  | —   ( 8.3±2.1)  |
| 2 acute†      | 80                                   | — (−3.3±0.8)  | —   (11.0±2.1)  |
| convalescent‡ | 160                                  | — ( 1.0±1.8)  | —   (−12.0±2.3) |
| 3 acute†      | 80                                   | — (12.3±0.5)  | —   ( 6.2±2.4)  |
| convalescent‡ | 40                                   | — ( 4.1±0.8)  | —   (10.7±2.9)  |
| 6§            | 20                                   | — (−1.4±2.5)  | + (31.0±4.3)    |
| 7§            | 160                                  | — (−8.7±1.4)  | + (24.0±2.1)    |
| 8§            | 40                                   | — ( 8.0±1.3)  | + (33.0±1.8)    |
| 9§            | 80                                   | — (11.3±1.9)  | + (39.4±1.2)    |
| 10§           | 320                                  | — (−3.4±2.0)  | + (33.6±0.7)    |
| 21§           | 160                                  | — (12.2±1.5)  | + (26.3±2.4)    |
| 28§           | 80                                   | — (12.3±3.1)  | + (32.7±2.8)    |
| 40§           | 40                                   | — ( 5.3±2.4)  | + (31.3±2.7)    |
| 51§           | 40                                   | — (13.2±1.4)  | + (31.4±1.6)    |
| 72§           | 20                                   | — (−2.4±1.1)  | + (23.1±2.3)    |

\*Leukocyte migration inhibition was determined using leukocytes (from a normal subject, case 7) whose migration is known to be inhibited by the EBV-antigen preparation in the presence of foetal bovine serum alone. % inhibition was 24.0±2.1. Mean of % inhibition ± s.e. was derived from eight replicate cultures. Less than 15% inhibition is marked as —, 20% or greater as +.

†Freshly-collected acute IM serum with EBV capsid antibody and Paul-Bunnell heterophil antibodies as in Table 1.

‡Freshly-collected convalescent IM serum with EBV capsid antibody but no Paul-Bunnell heterophil antibody.

§Serum collected from normal adults and stored at −20° C for 6–8 weeks before use.

||Indicates abrogation of expected leukocyte migration inhibition.

addition, a blocking factor specific to EBV was found in the acute and convalescent sera of these patients (Tables 2 and 3).

Haider *et al.*<sup>15</sup> have reported that *in vivo* CMI to tuberculin was apparently depressed in patients with acute IM. The absence of CMI to EBV in circulating leukocytes in the acute phase of the disease in the present study still allows the possibility that leukocytes mediating CMI to EBV are active outside the circulation. The development of CMI to EBV at the convalescent phase, however, as detected by leukocyte migration inhibition in the absence of autologous serum, suggests that a transient impairment of CMI function might have occurred.

**Table 3** Effect of sera from patients with IM on leukocyte migration inhibition induced by EBV or *E. coli*

| Serum              | Serum anti-EBV capsid antibody titre | Leukocytes from acute IM (case 4) | % Inhibition* from <i>E. coli</i> † | Leukocytes from convalescent IM (case 5) | % Inhibition* from <i>E. coli</i> † |
|--------------------|--------------------------------------|-----------------------------------|-------------------------------------|------------------------------------------|-------------------------------------|
| Equine alone       | 0                                    | 2±3.1                             | 28±0.5                              | 20±2.5                                   | 38±3.1                              |
| Normal human adult | 80                                   | −3±3.4                            | 38±1.0                              | 23±2.2                                   | 41±2.7                              |
| Case 4             | 160                                  | −2±3.4                            | 31±4.0                              | −5±1.0                                   | 36±3.3                              |
| Case 5             | 40                                   | −5±4.0                            | 29±3.1                              | 2±4.5                                    | 44±3.0                              |

\*% Inhibition is expressed as mean ± s.e. derived from a minimum of four replicate cultures.

†5.0×10<sup>7</sup> organisms ml<sup>−1</sup> prepared according to the method of Fimmel and Keast<sup>7</sup>.

It is not certain whether the blocking factor detected is part of normal negative feedback mechanisms involved in the immunity to virus infections, or whether it has an important role in the differential pathogenesis of diseases caused by EBV. The nature and the *in vivo* half-life of this blocking factor are unknown although we have shown that it was in the IgG fraction. Our experiments also indicate that it is not related to the EBV-capsid antibody, nor the Paul-Bunnell heterophil antibodies, as it is absent in normal sera with high level of EBV-capsid antibody (cases 7 and 10) and present in convalescent sera with no Paul-Bunnell heterophil antibodies. This could mean that the capsid antigen of EBV is not the antigen involved in the initiation of leukocyte migration inhibition, but as yet we have no direct evidence on this point.

Dameshek<sup>16</sup> has proposed that infectious mononucleosis, a benign lymphoproliferative illness, is a form of 'self-limiting' malignancy. It is plausible that CMI to EBV detected in these individuals is responsible for this 'self-limiting' expression. Considering the strong association of EBV with Burkitt's lymphoma and nasopharyngeal carcinoma it is important that CMI to EBV and the blocking factor be investigated in patients with these diseases at remission and recurrence.

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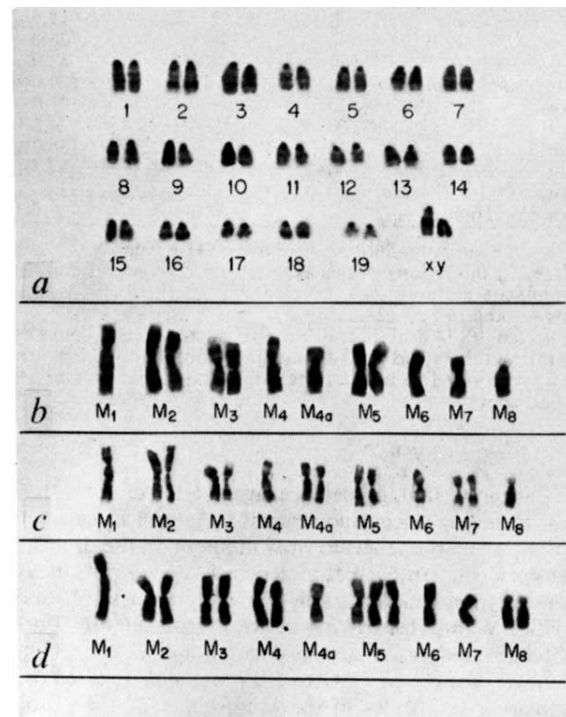
## Reversible appearance of a specific chromosome which suppresses malignancy

MALIGNANT cell transformation is associated with the appearance of abnormal chromosome patterns<sup>1</sup>. The degree to which malignant potential is expressed by transformed cells seems to depend on the balance between specific chromosomes responsible for the expression or suppression of malignancy<sup>2</sup>. Sachs *et al.*<sup>2-4</sup> have demonstrated a relationship between these chromosomes and both oncogenic activity and cell membrane characteristics in transformed and reverted cells. Benedict *et al.*<sup>5</sup>, identified specific chromosomes associated with either expression or suppression of malignancy in hamster fibrosarcoma cells; tumorigenicity in different clones was related to the balance of these chromosomes.

We examined the genetic constitution of an established tumour line, and the chromosome alterations which may develop, by karyotypic analysis of 122 metaphases in cells from cultures and tumour nodules. Table 1 shows that prolonged maintenance of MC-42 fibrosarcoma cells in tissue culture has no significant effect on the modal number of chromosomes, when compared with the number in cells from serially-transplanted tumours. Injecting these cultured cells into syngeneic hosts resulted in a slight decrease in the modal number of chromosomes, irrespective of the duration of *in vivo* propagation. In all cases, chromosome banding revealed a complete complement of normal mouse chromosomes (Fig. 1a) in both cultured and serially-transplanted tumour cells. Eleven to sixteen subtelocentric, submetacentric and metacentric marker chromosomes, were also present; the cells inconsistently gained normal mouse chromosomes, as well as a long telocentric chromosome (Figs 1 and 2).

The only consistent genetic alteration associated with the culturing of MC-42 cells appeared in the marker chromosome pattern which is characteristic of this tumour line (Figs 1 and 2). The eighth marker chromosome ( $M_8$ ) showed specific variations between cells from tumour nodules and those from cultures. All cells from subcutaneous tumours contained only one  $M_8$  chromosome. When the tumour was transferred to tissue culture, however, cells with two  $M_8$  chromosomes evolved,

first appearing (1 of 16 cells) in the 6th week of tissue culture propagation (Table 1). Four weeks later the proportion increased to 36% (4 of 11), and to 50% (11 of 22) after 12 and 17 weeks. Thereafter, the ratio of cells with one  $M_8$  to those with two remained 1:1, regardless of the duration for which the cells had been cultured (not shown in Table 1). None of the seven other groups of marker chromosomes observed in MC-42 tumour cells showed any consistent variation associated with *in vitro* propagation (Figs 1 and 2).



**Fig. 1** Chromosome banding patterns: *a*, Normal C3H/HeJ mouse cells. *b*, Marker chromosomes from a serially-transplanted tumour. Note the eighth marker ( $M_8$ ); all cells from growing MC-42 tumours contain only one  $M_8$  chromosome. *c*, Marker chromosomes from a cultured tumour cell containing one  $M_8$  chromosome. *d*, Markers from a cultured tumour cell containing two  $M_8$ . The tumour studied, a methylcholanthrene-induced fibrosarcoma (MC-42), had been propagated by serial transplantation in C3H/HeJ mice and had never been maintained in culture. The growth and immunogenicity of this tumour line have been described previously<sup>6,7</sup>. All tumours in this study were produced by subcutaneous inoculation of 20,000 viable cells (trypan blue exclusion) in McCoy's medium. Cultured cells were maintained in McCoy's 5a medium with 13% foetal calf serum, supplemented with penicillin, streptomycin and HEPES buffer. Cells were incubated in colchicine for 4 h, treated with trypsin and incubated in 1% sodium citrate for 20 min. They were then exposed to a fixative solution (acetic acid:methanol, 1:3) resuspended in 75% acetic acid and prepared for staining with 2% aceto-orcein. The method of trypsin/Giemsa R66 banding<sup>8</sup> of chromosomes was used.

Tissue culture clones composed entirely of cells having either one or two  $M_8$  marker chromosomes were grown by seeding cells from mixed cultures on to glass chips in Leighton tubes. Mass cultures containing equal proportions of cells with one and two  $M_8$  consistently produced a significantly greater number of clones of the one  $M_8$  variety. Multiple samples from clones, taken at periodic intervals, showed complete homogeneity with respect to either the single or duplicate  $M_8$  pattern; no clones older than 6 weeks were used. When cloned cells having two  $M_8$  were reimplanted into syngeneic hosts, the resulting tumour nodules were composed only of cells with one  $M_8$  chromosome (Fig. 2). Inoculations of cloned cells having one  $M_8$ , as well as inoculations of mass cultures, similarly produced tumours containing only cells with one  $M_8$  chromosome in every case. Cells with the duplicate  $M_8$  pattern did not appear *in vivo*, irrespective of the age of the cultures



inoculated, the size of the tumour nodule examined, or the duration of *in vivo* growth. When cells from various (mass) cultures were reimplanted into syngeneic hosts, the frequency of tumour formation was lower if the inoculum contained a greater proportion of cells with two  $M_8$  chromosomes (see Table 1). No other consistent alterations in chromosomes were observed when cloned cells were injected into mice, although an occasional gain of markers  $M_{4a}$  or  $M_6$ , or loss of an  $M_2$  or  $M_5$ , was inconsistently noted.

Therefore, although cells with two  $M_8$  seem as viable as those with one  $M_8$  *in vitro*, the capacity for tumour growth is expressed only by those which have lost the second  $M_8$ .

Lymphocytes from mice bearing serially-transplanted tumours (all containing one  $M_8$ ), compared with those from mice bearing tumours (of equal size) which had been produced by inoculating (9 week) mass cultured cells, are more cytotoxic to tumour cells in each of the four target cell groups (Fig. 3). This difference was not affected by the addition of serum from mice with the tumours. On the other hand, target cells were more efficiently lysed if they had been maintained (9 weeks) in tissue culture. Cells from clones with one  $M_8$  were destroyed as efficiently as those from mass cultures. In contrast, the cytotoxic effect of sensitised lymphocytes was significantly less when target cells with two  $M_8$  chromosomes were used.

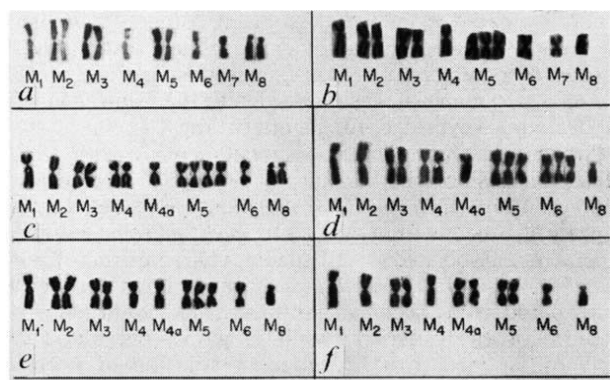


Fig. 2 Marker chromosome patterns: *a*, Tumour cell from a 10 week old mass culture. *b*, Cell from a tumour produced by injecting 20,000 (mass) cultured cells into a C3H/HeJ mouse. All cells in such tumours contain only one  $M_8$  chromosome. *c*, Tissue culture clone (c1-7) containing only cells with two  $M_8$ . *d*, Tumour nodule grown by injecting cells from the cultured clone c1-7 (shown in *c*). *e*, Tissue culture clone (c1-2) containing only cells with one  $M_8$ . *f*, Tumour produced by subcutaneous inoculation of cells from clone c1-2 (shown in *e*).

The decreased ability of mass cultures of tumour cells containing both cells with one and two  $M_8$  to sensitise lymphocytes, together with the significantly lower cytotoxicity of target cells containing two  $M_8$ , may reflect the failure of cells with two  $M_8$  to express their tumour-associated antigens. The lower cytotoxicity does not seem to be directly related to the culturing process, as cells from mass cultures and from clones with one  $M_8$  were more efficiently lysed than cells from serially-transplanted tumours.

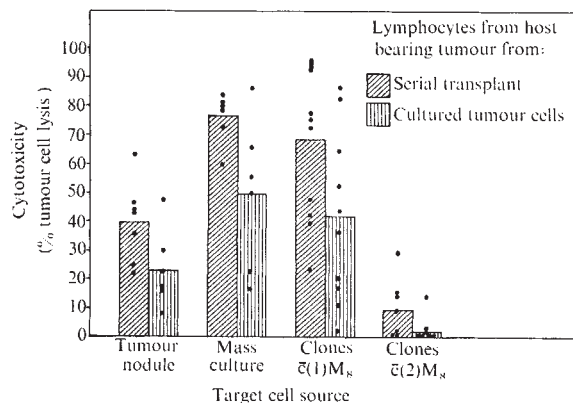
Giemsa banding of chromosomes (Fig. 1) shows that the  $M_8$  has no bands on the short arms, suggesting that this portion of the chromosome may be from the normal mouse chromosome 19. The centromere is heavily stained and the long arms contain three prominent, moderately-stained bands in the mid-region, indicating that this portion may be an unaltered number 7 chromosome. Karyotypic studies of cells containing two  $M_8$  consistently suggest that the  $M_8$  is derived from a deleted number 19 chromosome, translocated on to a number 7. All MC-42 cells contain a complete complement of normal chromosomes together with an inconsistent number of additional chromosomes.

Multinucleated cells were present in all cultures before the duplicate  $M_8$  pattern occurred. None of the cells displayed asynchronous DNA synthesis (which might indicate cell fusion) after exposure to  $^3H$ -thymidine for 5 h. This suggests that multinucleation, chromosome ploidy and the evolution of MC-42 cells with two  $M_8$  chromosomes may be the result of mitotic abnormalities, rather than of cell fusion.

Our data, therefore, show a strong association between the appearance of a second  $M_8$  marker chromosome in MC-42 fibrosarcoma cells and the suppression of malignant characteristics. Sachs *et al.*<sup>1</sup> have found that the *in vitro* propagation of polyoma virus-transformed cells may favour the appearance of chromosomes which suppress malignant characteristics. By fusing highly malignant ascites tumour cells with non-malignant cells, Harris *et al.*<sup>10</sup> produced both tumour cells in which malignancy and the expression of histocompatibility antigens were suppressed; a loss of chromosomes from these hybrid cells was associated with recovery of the ability to grow progressively *in vivo*. These reports are consistent with our data, in that cultures of the MC-42 fibrosarcoma developed variants which appeared less tumorigenic and less immunogenic, and which lost a chromosome when malignancy was expressed. The MC-42 tumour line, however, contains the only example of a suppressor chromosome (the second  $M_8$ ) which is paired with a morphologically identical chromosome ( $M_8$ ) that alone has no apparent direct influence on the suppression of malignancy. In polyoma virus-transformed hamster cells<sup>11</sup>, where the chromosome group which suppresses malignancy has been identified, there seems to be a direct relationship between the number of suppressor

Table 1 Chromosome variations in MC-42 fibrosarcoma cells

| Cell source                       | Tumour diameter (mm) | Age of culture (weeks) | Cells with chromosome number = |       |       |       |       |       |       |       |     |           | No. of cells containing: |      | No. of mice with tumour/ no. of mice injected |
|-----------------------------------|----------------------|------------------------|--------------------------------|-------|-------|-------|-------|-------|-------|-------|-----|-----------|--------------------------|------|-----------------------------------------------|
|                                   |                      |                        | 48-51                          | 52-53 | 54-55 | 56-57 | 58-59 | 60-61 | 62-63 | 64-72 | 106 | (1) $M_8$ | (2) $M_8$                |      |                                               |
| Serial transplant                 | 12                   | —                      | 1                              |       |       | 3     | 5     | 2     |       |       |     | 11        | 0                        |      |                                               |
|                                   | 34                   | —                      |                                |       |       |       |       | 3     | 1     |       |     | 4         | 0                        |      |                                               |
| Tumour culture                    | —                    | 6                      |                                | 1     |       | 3     | 6     | 3     | 1     | 2     |     | 15        | 1                        | N.D. |                                               |
|                                   | —                    | 10                     |                                | 4     | 1     | 5     | 4     |       |       |       | 1   | 11        | 4                        | 6/6  |                                               |
|                                   | —                    | 12                     |                                |       |       | 4     | 5     | 1     |       |       |     | 5         | 5                        | 3/6  |                                               |
|                                   | —                    | 17                     |                                | 2     |       | 9     | 1     |       |       |       |     | 6         | 6                        | 3/6  |                                               |
| Cultured cells ( <i>in vivo</i> ) | 4                    | —                      |                                |       | 1     | 2     |       |       |       |       |     | 3         | 0                        |      |                                               |
|                                   | 17                   | —                      | 1                              | 2     | 4     | 5     | 3     | 1     |       |       |     | 16        | 0                        |      |                                               |
|                                   | 20                   | —                      | 1                              |       | 2     | 1     |       |       |       |       |     | 4         | 0                        |      |                                               |
|                                   | 25                   | —                      |                                |       | 2     |       |       |       |       |       |     | 2         | 0                        |      |                                               |
|                                   | 26                   | —                      |                                | 2     | 2     | 1     |       |       |       |       |     | 5         | 0                        |      |                                               |
|                                   | 28                   | —                      | 2                              | 2     | 6     | 2     |       |       |       |       |     | 12        | 0                        |      |                                               |
|                                   | 29                   | —                      |                                | 1     | 2     |       |       |       |       |       |     | 3         | 0                        |      |                                               |
|                                   | 31                   | —                      |                                | 1     | 6     | 2     |       |       |       |       |     | 9         | 0                        |      |                                               |



**Fig. 3** Ability of lymphocytes from mice administered serially-transplanted or (mass) cultured tumour cells to lyse target cells from either serially-transplanted tumours, mass cultures, clones of cells with one  $M_8$  or those with two  $M_8$ . Lymphocytes were purified (Ficoll-hypaque separation) from the spleens of animals bearing palpable tumours and the cytotoxicity assay was performed according to the method described previously<sup>8</sup>. Target cells were plated in Falcon 3040 Microtest II wells and the cells directly counted 24 h later. Target cells were then exposed to lymphocytes for 2 d, washed with saline and stained with crystal violet for final counting.

chromosomes and the degree of suppression. Furthermore, a chromosome group responsible for the expression of malignancy has been identified in that tumour, the number of such chromosomes in cells of any clone being proportional to the degree of malignancy of that clone. When cells from clones of suppressed polyoma virus-transformed cells are reimplanted into syngeneic hosts, the tumours produced contain fewer suppressor chromosomes and more chromosomes for the expression of malignancy. Careful examination of MC-42 tumour cells failed to demonstrate a specific chromosome group responsible for the expression of malignancy.

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## Isolation of *Bacillus anthracis* from an aborted bovine foetus

We report here the presence of virulent anthrax bacilli in aborted tissues. This is significant in that it suggests that the foetus becomes infected during the bacteraemia/pyrexia phase of the dam, and that the bacilli continue to multiply in and cause the death of the foetus despite the recovery with treatment in the parent cow. Since abortion may be a sequel to the infection in pregnant animals which survive, the products of such abortions must be regarded as potential vectors of anthrax both for man and other animals.

In Spring 1974 two cows died within 24 h of each other and anthrax was confirmed in each case. Control measures included the immediate vaccination of the remainder of the herd with a proprietary spore vaccine. Anthrax Spore Vaccine (Living), (Wellcome). The following day a cow became ill, having a rectal temperature which rose to 41.1°C. Antibiotic therapy was instituted and the animal recovered within 48 h. But 13 d after vaccination this cow aborted a foetus and placenta within an isolation box. In keeping with the Brucellosis Incentives Scheme rules, serum, milk and the products of abortion were forwarded to the local Veterinary Investigation Centre. The gestational age of the foetus was determined as 120 d, but apart from softening of the liver no gross pathological changes were seen in either the foetus or the placenta which might have indicated the possible presence of anthrax. Examination of smears prepared from foetal stomach contents and placental cotyledons revealed chains of encapsulated bacilli; overnight cultures prepared from similar sites were suggestive of *Bacillus anthracis* and material was forwarded to the Central Veterinary Laboratory, Weybridge, for identification.

Two guinea pigs inoculated subcutaneously with 1 ml of a saline suspension of the culture ( $\sim 1 \times 10^6$  organisms) and 1/100 dilution of a saline suspension ( $\sim 1 \times 10^4$  organisms) respectively died within 48 h. On *post mortem* examination hyperaemia associated with oedema, characteristic of anthrax, was observed and *Bacillus anthracis* was isolated in pure culture from blood and oedema. Anthrax was confirmed by the presence of encapsulated bacilli in guinea pig blood smears fixed and stained with 1% aqueous solution of polychrome methylene blue. The vaccine strain is seldom lethal for guinea-pigs and is not encapsulated if present in the blood of inoculated guinea pigs<sup>1</sup>.

Two vaginal swabs and a faeces sample, taken from the cow 3 d after abortion, did not yield anthrax bacilli on bacteriological examination at the Central Veterinary Laboratory.

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## Erratum

In the article "Precocious development of detoxicating enzymes following pituitary graft" by G. J. Wishart and G. J. Dutton (*Nature*, **252**, 408; 1974) line 3 in paragraph 10 should read '... similar weight from 2-d chicks down to 18-d embryos,' and not as printed.



# matters arising

## Evolution of the Tasman Sea

WE do not accept Hayes and Ringis' "conclusive evidence" that the central Tasman Sea evolved by symmetrical spreading from a median north-west trending ridge between 80–60 Myr ago, for several reasons.

First, their 'ridge' and 'fracture zones', which are mantled by sediments, have no bathymetric expression.

Second, they ignore the only conspicuous topographic features within the limits of their magnetic anomaly

pattern. These are the seamounts and knolls respectively north and south-west of Gascoyne Guyot. Both trends are oblique to their magnetic grain and fracture direction (Fig. 1).

Third, their seismic data are said to "reveal a distinct basement ridge" blanketed by acoustically transparent sediments which "thin subtly near the crest of the buried ridge". We contend that only their profile X could be described thus, and its "axial rift" is equivocal.

Fourth, they ignore an elongate area of thin sediments<sup>2</sup> which is coaxial

with the knolls south-west of Gascoyne Guyot (Fig. 1), but which is unrelated spatially to the 'ridge' and 'fracture zones'.

Fifth, they are prepared to consider that spreading "was much slower in the northern area than in the southern area", but this is contrary to the anomaly spacings, and therefore to the identifications, which they adopt.

Sixth, they maintain that limited subduction along the Tasman margin of Australia 80–60 Myr ago cannot be excluded. Yet no rocks of orogenic character on this margin are younger than Triassic and all are related to a Palaeozoic–Mesozoic tectonic regime expressed in a north-north-west continental grain which is cut obliquely by the present margin. The only post-Triassic volcanics on this margin are non-orogenic and are less than 55 Myr old<sup>3</sup>.

Seventh, their pre-Tasman reconstruction of Australia/Antarctica and the New Zealand Plateau involves a misfit (gaps plus overlaps) which is equivalent to half the area of the latter. Furthermore, they reduce the potential misfit considerably by eliminating the Alpine Fault, although most studies indicate that major movement on it occurred during the early Cretaceous<sup>4</sup>.

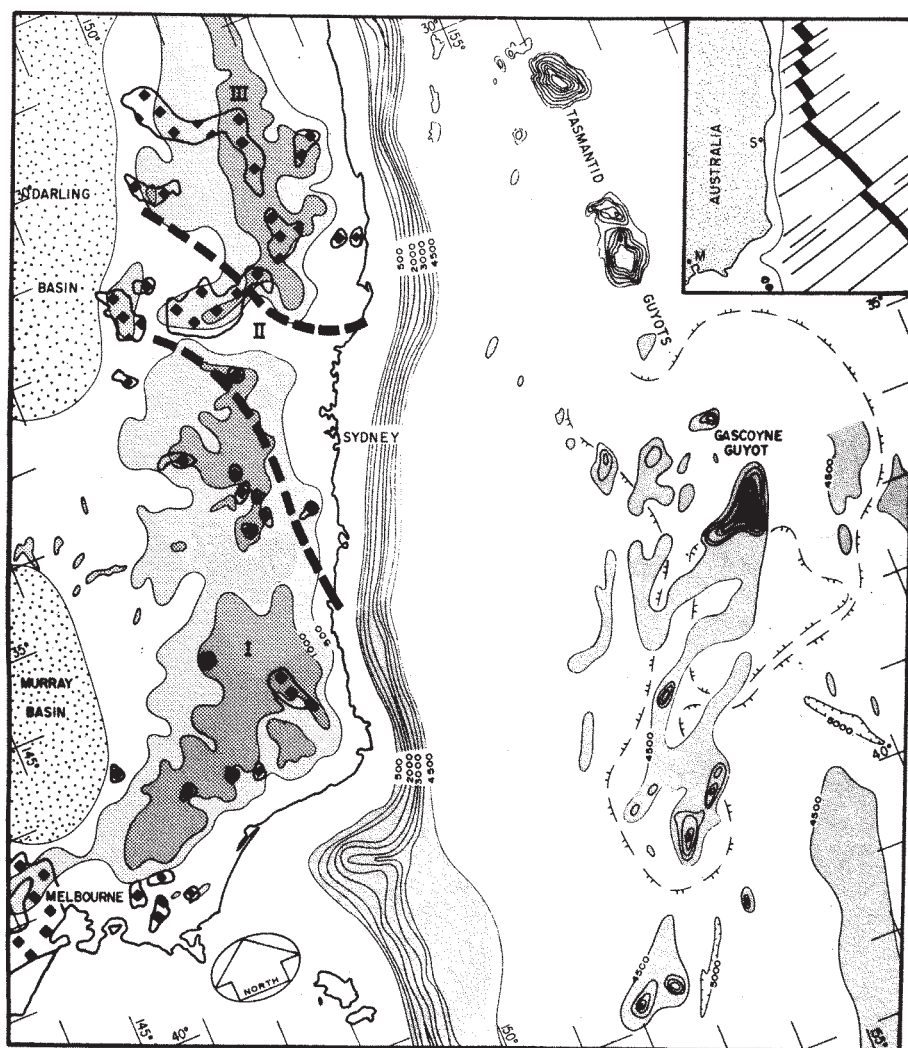
Eighth, their hypothesis cannot account for the NE–SW orientation (oblique to the trends of their 'ridge' and 'fracture zone'; Fig. 1) of the dominant topographic elements of the Tasman margin, centred on Sydney.

Finally, their hypothesis cannot account for recurrent Cainozoic basaltic volcanism on Australia's Tasman margin<sup>3</sup> which entirely post-dates their postulated termination of Tasman dilation 60 Myr ago.

A more extensive discussion of these points can be obtained from us.

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**Fig. 1** Tasman margin of mainland Australia. Contour interval at 500 m. Diamond stipple, Cainozoic volcanics; roman numerals, geological provinces of the pre-Tasman tectonic regime: I, early Palaeozoic; II, late Palaeozoic–early Mesozoic; III, mid-Palaeozoic to early Mesozoic. Broken lines, approximate 600 m sediment isopach, with barbs indicating direction of thickening. Bathymetry after Hayes and Connolly<sup>5</sup> and Connolly<sup>6</sup>. Sediment isopach from Houtz *et al.*<sup>2</sup>. Inset shows spreading ridge and fracture zone trends of Hayes and Ringis<sup>1</sup>.

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## Red Sea spreading

GIRDLER and Styles<sup>1</sup> cite geological evidence<sup>2</sup> from the Gulf of Suez area favouring the idea that a Carboniferous Red Sea depression existed. Various views have been put forward concerning the age of the Suez rift structures<sup>3-5</sup>. Some authorities believe block faulting and rifting does not predate Oligocene<sup>6</sup>; others have isopached a post-crystalline Basement Complex to pre-Permian linear trough<sup>7</sup>; and some believe in a Mesozoic trough<sup>8</sup>.

Even if definite proof were available publicly of a Carboniferous trough this would not necessarily prove that a trough of this age existed in the Red Sea area, because the Suez structures are part of a failed arm system<sup>8,9</sup>; the Red Sea structures are part of an active spreading ridge system and the Aqaba-Dead Sea structures part of an active transform system. As such the three arms have had interlinked but separate geological histories<sup>8</sup>.

There is clear evidence in the southern Red Sea area, however, of major rifting before the Early and Medial Eocene proposed by Girdler and Styles<sup>1</sup>. A pre-Mesozoic (?pre-mid Jurassic) trough some 500 km long; up to 150 km wide and containing up to 7.5 km of sediments in the Mandera region of Somalia extends from coastal Tanzania to Afar<sup>10</sup>.

This trough as roughly mapped intersects the Cainozoic RRR Afar junction. Some implications are: first, that the Somalia trough may be part of an older failed rrr 'Karoo' junction, with the other failed arms buried beneath the sediments of the southern Red Sea and Gulf of Aden; second, the evolution of the Cainozoic Nubian - Somalian - Arabian plate relationships must be viewed with this in mind; and third, that the Mesozoic African plate may have rotated anticlockwise after the attempted 'Karoo' disruption (crustal thinning, necking, troughing and so on) so enabling the Cainozoic East African Rift to develop over the 'Karoo' mantle plume system.

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DR GIRDLER AND MR STYLES REPLY—WHITEMAN'S views on the formation of the Red Sea are well known. He considers<sup>1</sup> that "downwarping has been the major force in shaping the Red Sea depression" and he objects to any major crustal separation with the evolution of oceanic crust.

His objection to a Carboniferous Red Sea Depression is puzzling since in the literature he has been an advocate of this. In the summary of his paper *Formation of Red Sea Depression* he states "A depression existed in the northern part of the region in Carboniferous times . . ." and on p. 237 we find "In the writer's (Whiteman's) view the Red Sea has been an area of subsidence for a large part of post-Cambrian time." We respect that this is a view held by some geologists and mentioned it in passing<sup>2</sup>. We were careful to put a question mark<sup>3</sup> in our Fig. 7 and it should be clear from our Figs 5 and 6 that it is unimportant to our arguments.

We have been interested in the postulated 'ancestral pre-Jurassic trough' running from the Southern Red Sea across the horn of Africa to intersect the Tanzania coast<sup>3,4</sup> but at the moment it seems unrelated in time and space to the seafloor spreading discussed.

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<sup>3</sup> Beltrandi, M. D., and Pyre, A., in *Sedimentary Basins of the African Coast*, 159-178 (Assoc. African Geol. Surv., 1973).

<sup>4</sup> Black, R., Morton, W. H., and Hailu, T., *Nature*, **248**, 496-497 (1974).

## Solubility of oxygen-nitrogen mixture in water

MAHARAJH and Walkley<sup>1</sup> have reported that mixtures of gases containing oxygen do not behave independently when they are dissolved in water, and as a consequence Henry's law may be

deviated from by as much as 30%. Liss and Slater<sup>2</sup> found that the amount of oxygen dissolved in water from an air mixture at 25°C and atmospheric pressure obeyed Henry's law within their experimental limits (4%). Wilson<sup>3</sup> has reviewed some of the various measurements of oxygen solubility in water at different partial pressures and showed that Henry's law is generally accurate in predicting the amount of gas dissolved to within a few per cent. A thermodynamic analysis<sup>4</sup> of Maharajh and Walkley's results showed that the large negative deviation from Henry's law (maximum at about a 1:1 nitrogen to oxygen ratio) leads to an absurd value for the Henry's law constant.

There have been many reports of the solubility of gas mixtures in water, and in most cases these may be shown to be in agreement with Henry's law. Glasstone<sup>5</sup>, using Winkler's data<sup>6</sup>, has demonstrated that the solubilities of oxygen, nitrogen and argon each multiplied by their atmospheric partial pressure may be used to calculate the solubility of air in water to within 1%. We have calculated the hypothetical solubility of an air mixture<sup>7</sup> of oxygen, nitrogen, argon and carbon dioxide, using smoothed literature values for the solubilities of the individual gases<sup>8,9</sup>. The resulting solubility is 4% higher than that reported by Winkler<sup>6</sup>. Behnke<sup>10</sup> measured the solubility of a mixture of argon and nitrogen in water, and his value is 5% above the value calculated from Henry's law and using the recommended literature values for the solubilities of argon and nitrogen<sup>8</sup>.

We have measured the solubilities in water of nitrogen, oxygen, and a mixture of oxygen (49.5 mol %) and nitrogen (50.5 mol %) at atmospheric pressure and 25°C. The procedure used for the solubility measurements has been described previously<sup>11</sup>, and the precision of these measurements is  $\pm 1\%$ . The experimentally determined solubilities expressed as mole fractions (at one atmosphere partial pressure of gas) are given in Table 1.

The value predicted for the mixed gases on basis of Henry's law is  $0.1711 \times 10^{-4}$ , which is 3% lower than the experimental value (average,  $0.1770 \times 10^{-4}$ ). These results are conclusive evidence that Henry's law is not deviated from

**Table 1** Solubilities as mole fractions  $\times 10^4$ . Literature<sup>8</sup> values are given in parentheses

| Gas                            | Experiment                         | Calculated |
|--------------------------------|------------------------------------|------------|
| N <sub>2</sub>                 | 0.1166<br>0.1166 (0.119)           | —          |
| O <sub>2</sub>                 | 0.2261<br>0.2251<br>0.2284 (0.231) | —          |
| O <sub>2</sub> /N <sub>2</sub> | 0.1775<br>0.1779<br>0.1757         | 0.1711     |



by as much as Maharajh and Walkley claim. It appears that if anything, the solubility of oxygen in water is enhanced to a small extent by the presence of nitrogen rather than lowered by it.

We have had considerable experience in determining gas solubilities by gas chromatography (the technique Maharajh and Walkley used). It is extremely difficult to attain precisions better than 3% by this method. Indeed, in our judgment, the precision of other workers may actually be as poor as  $\pm 10\%$ . We suggest that the results reported by Maharajh and Walkley are due to inherent difficulties in the gas chromatographic approach.

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## Redshift during Pioneer-6 solar occultation—unexplained or predicted

THE recent detailed analysis<sup>1,2</sup> of Pioneer-6 data<sup>3</sup> demonstrated, under controlled conditions, the existence of the redshift effect which we predicted<sup>4–6</sup>. The explanations proposed by us for certain anomalous redshifts were based on the simple fact, that a moving inhomogeneous medium results in frequency shift and line broadening<sup>4,5</sup>. (But there is no close correlation between line broadening and frequency shift.) Therefore the search for highly specific or hypothetical explanations for redshift effects, observed on or around the Sun, seems to be unjustified.

The Pioneer-6 frequency residuals could be reproduced well (Fig. 1), without any effort to reach a better agreement between

measured points and theoretical curve. In computing the theoretical curve, the method described in refs 4–6 was used. In the given range of heliocentric distances the particle density was taken proportional to  $r^{-3}$  and the velocity of the medium was decreased in the vicinity of the Sun. Unfortunately the original data of Pioneer-6 were not available to us, so we were compelled to use the data

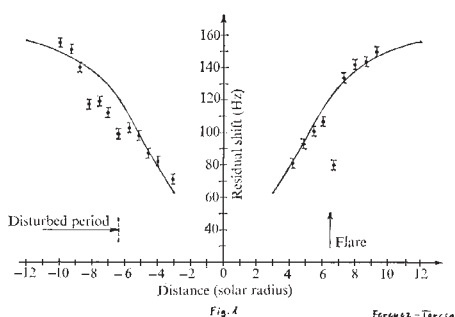


Fig. 1 Residual frequency shift<sup>1</sup> and computed curve, corresponding to the moving medium around the Sun, as a function of the minimum distance of Pioneer-6 2,292 MHz radio signal to the centre of the Sun.

published in refs 1–3. From these, however, one cannot establish exactly the zero-line of frequency residuals. Therefore the measured points and the theoretical curve were tied together on the basis of the measured data, referring to  $r = 5 R_{\odot}$ , known satisfactorily from the Taurus A experiments<sup>4</sup>.

Furthermore, the overall picture of solar activity exhibits a correlation with deviations from the theoretical curve, and the effect of enhanced solar activity appears also in the asymmetric bandwidth pattern (ref. 2, Fig. 1b).

With special respect to the opportunities afforded by the space shuttle, it would be expedient in the future to measure accurately the solar disk and corona (the latter by occultational methods) for frequency shift and line broadening in different regions of the electromagnetic spectrum.

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## Spatial distribution of cometary outbursts

PATASHNICK *et al.*<sup>1</sup>, in reproducing figures of the position of comets at the times of cometary outbursts from the papers of Pittich<sup>2,3</sup>, have omitted the outbursts of comet P/Schwassmann-Wachmann (1) (1925 II) which have been observed regularly since 1927 (ref. 2). Richter<sup>4,5</sup> concluded that the outbursts of the latter were essentially similar to those of 12 other comets. The omission of these data is therefore unjustified.

Figure 1 is a histogram of the number of observed cometary outbursts  $N_0$  in the interval  $R$  to  $R+0.25$  AU as a function of  $R$ , the distance between the comet and the Sun at the time of outburst. Three regions are apparent:

- (a)  $R < 1$  AU. The increase of  $N_0$  with  $R$  is simply a reflection of the distribution of cometary perihelion distances<sup>6</sup>.
- (b)  $1 < R < 5.5$  AU. A minimum deviation fit gives

$$N_0 = (24 \pm 4) R^{-(2.2 \pm 0.1)}$$

Contrary to ref. 1, it is thought that here  $N_0(R)$  is determined chiefly by observational selection, the ratio between detected and undetected outbursts decreasing as the Earth-comet distance increases.

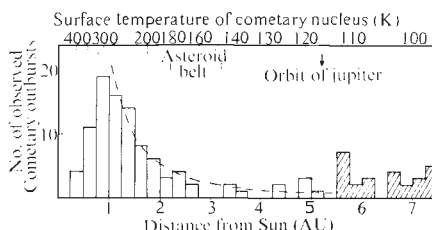


Fig. 1 Number of observed cometary outbursts as a function of distance from the Sun  $R$ . Hatched regions, Comet P/Schwassmann-Wachmann (1); White regions, other comets; dashed curve, minimum deviation fit to the data in  $1 < R < 5.5$  AU region.

(c)  $R > 5.5$  AU. These data originate *in toto* from comet P/Schwassmann-Wachmann (1) containing outbursts from two complete orbits. There is no clear variation of  $N_0$  with  $R$ . It is therefore concluded that the probability of outburst occurrence for the other comets is also independent of  $R$ , at least for  $R < 7.5$  AU, and similarly independent of temperature for  $T > 115$  K (the surface temperature of the cometary nucleus is shown as the upper abscissa in Fig. 1, this being calculated assuming that the cometary nucleus has an albedo of 0.6 and is a rapidly rotating sphere). This rules out the three temperature-dependent theories of outburst production—Whitney<sup>7</sup> requiring  $T > 130$  K to produce pockets of vaporised methane, Donn and Urey<sup>8</sup> requiring  $T > 148$  K to produce the explosive  $\text{NH}_4\text{N}_3$  and Patashnick *et al.*<sup>1</sup>

needing  $T > 140$  K for the phase transition between amorphous and cubic ice. This latter theory also requires amorphous ice to have the suspiciously high density of  $2.3 \text{ g m}^{-3}$ , implying an O-O distance of  $\sim 2.0 \text{ \AA}$  compared with the more normal  $2.8 \text{ \AA}$ . None of these can account for the outbursts of P/Schwassmann-Wachmann (1).

Three theories remain—tidal disruption by Jupiter, asteroidal collision and collision with boulders<sup>9</sup> (mass  $\sim 10^8 \text{ g}$ ). Pittich<sup>2</sup> has ruled out the first two, leaving only the boulders.

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## Changes in the latitude of the climatic zones of the Northern Hemisphere

IN a discussion of the drought in the Sahelian Zone of Africa it has been suggested<sup>1</sup> that the drought is associated with a shift of the climatic zones of the Northern Hemisphere towards the equator. An analysis by Lamb was quoted as evidence for this shift, but Lamb's analysis was mainly related to the

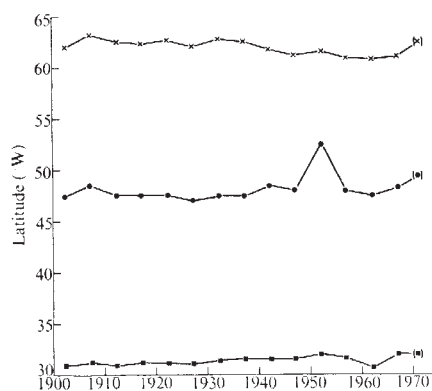


Fig. 1 Average latitudes of climatic zone parameters in the North Atlantic (whole year). Five year means except the last plot which is 1970–1973. ×, Subpolar flow; ●, westerly maximum; ■, subtropical high.

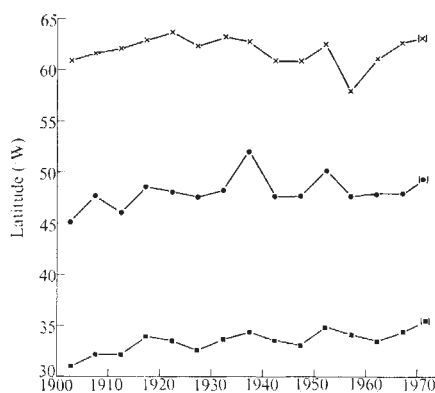


Fig. 2 Average latitudes of climatic zone parameters in the North Atlantic (summer). Five year means except the last plot which is 1970–37. ×, Subpolar flow; ●, westerly maximum; ■, subtropical high.

subpolar pressure minimum, and the latitude of the subtropical pressure maximum would seem to be more relevant to the Sahelian zone.

Using values of sealevel pressure stored on magnetic tape in the form of a grid, the latitudes of the subpolar minimum of pressure, the subtropical maximum of pressure and the latitude of strongest westerly winds were calculated. Non-overlapping periods of 5 yr from 1900 to date were used, both for the northern hemisphere and the North Atlantic region ( $10^{\circ}\text{W}$  to  $60^{\circ}\text{W}$ ). Figure 1 shows these three parameters for the Atlantic region for the whole year and Fig. 2 for the Atlantic for the summer (June, July and August).

The data for the whole year confirm Lamb's analysis in indicating a slight trend of the subpolar pressure minimum towards the equator but show no trend or even a slight one towards the pole for the maximum of the westerlies and the subtropical pressure maximum. The data for summer lead to broadly the same conclusion, except that the trend of the subtropical pressure maximum towards the pole is stronger. The results for the whole hemisphere are similar. What

is particularly notable is the trend of all the parameters towards the pole for the whole year and for the summer over the past three 5 yr periods while the Sahelian rainfall has been decreasing. This makes the hypothesis that the present Sahelian drought is due to a shift of the climatic zones towards the equator scarcely tenable.

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## Role of arginase in the epidermis

THE article by Oka and Perry concerning the role of arginase in the mammary gland<sup>1</sup> suggests an explanation for the high levels of this enzyme in the skin<sup>2,3</sup>. This tissue resembles mammary gland in that the remaining enzymes of the urea cycle seem to be absent.

A large body of work exists regarding the stimulation of epidermal proliferation as a result of wounding. Such stimulation is preceded by the induction of ornithine decarboxylase and the production of putrescine and spermidine under the influence of the epidermal growth factor<sup>4,5</sup>. Thus, it seems possible that the function of arginase, at least in the epidermis, is to provide a reservoir of ornithine for the production of spermidine. This idea is consistent with the observations that cutaneous lesions associated with pathological proliferation of rete cells, such as psoriasis and the common wart<sup>2</sup>, or following experimental application of carcinogenic substances<sup>6</sup>, show greatly increased arginase activity.

Of course it is possible, as Oka and Perry point out, that arginase may also be involved in the production of proline, although this seems less likely to be of significance in the epidermis than in the dermis.

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## Matters arising

Matters Arising is meant as a vehicle for comment and discussion about papers that appear in *Nature*. The originator of a Matters Arising contribution should initially send his manuscript to the author of the original paper and both parties should, wherever possible, agree on what is to be submitted. Neither contribution nor reply (if one is necessary) should be longer than 300 words and the briefest of replies, to the effect that a point is taken, should be considered.

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# reviews

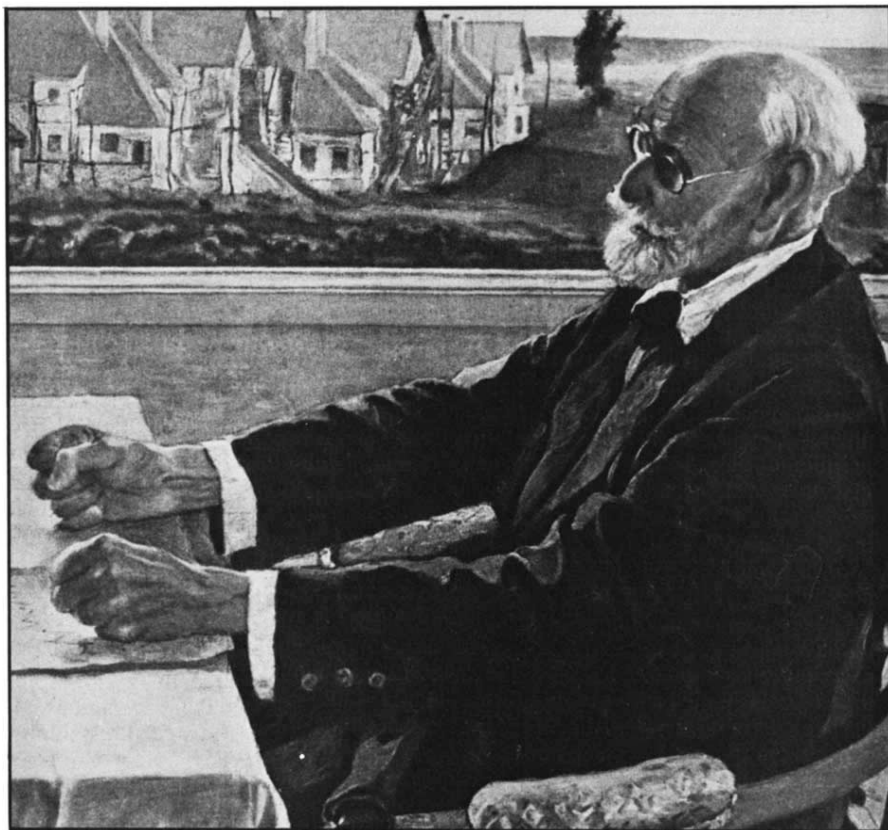
## Pavlovian views in Soviet science

*Biology and Neurophysiology of the Conditioned Reflex and its Role in Adaptive Behaviour.* By P. K. Anokhin. Pp. xvi+574. (Cerebrovisceral and Behavioural Physiology and Conditioned Reflexes.) (Pergamon: Oxford and New York, June 1974.) £17.00.

FEW historical figures command the attention of working scientists. Pavlov, however, remains important, not only for the obvious historical reason that he opened up a new and fruitful field of study, but for the eminently practical reason that many of his detailed findings and behavioural analyses are as valid today as when they were first formulated. Among western students of animal learning, interest in classical or Pavlovian conditioning is probably greater today than it has ever been: it has become increasingly clear that such conditioning is of wider scope and greater importance than has been suggested by the traditional American emphasis on instrumental learning and the law of effect.

It is interesting, therefore, to see how the Pavlovian tradition has fared in the Soviet Union, and one may welcome a major statement by one of Pavlov's students, even if blenching at the price one is asked to pay for it. Even more welcome might have been a statement by a psychologist of a younger generation, but it still seems that only those who sat at the feet of the master can hope to have their books translated into English. Can Soviet psychology really be so patriarchal, or is this a reflection on the policy of western publishers?

It must be said at the outset that this is not a book for casual browsing. It is very hard going. A major problem is that, as is here amply confirmed, Soviet and western students of conditioning have taken over quite different facets of Pavlov's work. Pavlov did two things: first, he studied the behavioural facts about the new conditional reflexes he had discovered by accident, and attempted to elucidate the laws governing their acquisition, extinction, generalisation, and so on; second, he attempted to infer the physiological



*Ivan Pavlov—still the Soviet patriarch?*

mechanisms responsible for these behavioural laws. Although Pavlov did not draw a sharp distinction between these tasks, it is important to recognise that they do indeed differ. Western behaviourists, at any rate, have followed the former programme of research; but in the Soviet Union, where the study of conditioning is described as the physiology of higher nervous activity, it is the latter interest that has prevailed.

Even if it is accepted that the eventual goal of psychology is to understand behaviour in terms of the functioning of the nervous system, it seems likely that this goal will be attained only after the establishment of valid behavioural laws, and the achievement of some insight into the general logic of the system which produces that behaviour. Although the neurophysiological knowledge which Anokhin can draw on is immeasurably more sophisticated than that available to Pavlov 50 years ago, it is not clear that this increase in knowledge has advanced our understanding of conditioning. Anokhin's use of neurophysiological evi-

dence, at any rate, is often distinctly peculiar. A good example is his discussion of the "accursed problem" (Pavlov's description) of inhibition. Behaviourally, the term inhibition is used in several ways: an inhibitory stimulus, for example, is one which signals the omission of the unconditioned stimulus; an inhibited response is one which does not occur in a situation in which it has occurred in the past. In neurophysiology, the term is used in a different set of ways, here most often to refer to the blocking of neural transmission ascribed to post-synaptic hyperpolarisation. Anokhin has no qualms about using this neurophysiological concept to dictate a number of psychological conclusions. Thus, since physiological inhibition "exists only so long as the excitation that gave rise to it exists," inhibitory conditioning can occur only in response to prior excitatory conditioning. Again, since physiological inhibition is a "purely local process" which "always remains at its point of origin," we can reject Pavlov's theory of inhibition which assumed that inhibitory stimuli

themselves acquired new, inhibitory properties, for that requires the assumption that inhibition is initially localised in sensory cortical areas, and would then have to be propagated to the cortical representation of the unconditioned reflex. Indeed, the fact that physiological inhibition does not irradiate over the cortex is used by Anokhin to establish an even stronger conclusion: since the effects of inhibitory conditioning to one stimulus do generalise to similar stimuli, such conditioning cannot, in fact, be inhibitory at all; its effects must be caused by the excitatory conditioning of an antagonistic motivational state.

Most western psychologists, I think, would agree that this type of argument was radically misconceived. What they will find worse, is the way Anokhin continually shifts back and forth between behavioural and physiological evidence, with no clear recognition of the difference between the nature of the evidence in the two cases. With some effort, one can discern the similarities between Anokhin's behavioural arguments and those familiar to western psychologists. Thus, Anokhin's general analysis stresses the importance of a feedback principle (the action acceptor), whereby discrepancies between behaviour and goal are registered and serve to initiate correcting action. Inhibitory learning is then said to arise when the organism detects an adverse discrepancy between the actual and expected outcome of a trial (as the omission of expected food), and this generates an aversive motivational state which suppresses the organised appetitive activity originally conditioned by the presentation of food. There is an obvious resemblance to the version of frustration theory proposed by Spence, Amsel and others, and Anokhin occasionally adduces behavioural evidence in support of his account, which is gratifyingly similar to that advanced in support of frustration theory. But this is interspersed with neurophysiological evidence, and separated by chapters of neurophysiological analysis, the behavioural implications of which are at best obscure, and at worst patently nonexistent.

As its title suggests, the book is concerned not only with the analysis of conditioning and learning, but also with the biological significance of unconditioned reflexes, and the nature of adaptive systems. Occasional passages in these chapters ring true to a western ear, but it is hard to find many examples of sustained argument or of close interplay between data and theory. It seems depressingly insular to say so, but if there is anything for us to learn from Soviet psychology, it is not apparent from this book.

N. J. Mackintosh

## Book of the moth

*The World of Moths.* By Michael Dickens and Eric Storey. Pp. 127. (Osprey: London, 1974.) £2.25.

In a world that contains over 100,000 species, authors who must restrict an introductory book to cover only 100 species have a difficult task. Although there may be a temptation to choose only British species, I am delighted to find that this small, unpretentious book makes those new to the subject, especially young people, immediately aware of what exists in the tropics. It dispels the illusion that moths are "dirty brown things" and although 'professionals' will argue that but a few families are represented, with emphasis only on hawk moths (30 species) and silk moths (39 species), I think this is a delightful 'starter's book' with a whole page of useful information devoted to each species illustrated. The coloured photographs are splendid and I am sure that, at its modest price, the book will be a favourite Christmas gift.

E. R. Laithwaite

## Radiation, ecology, and Soviet concern

*Radioecology.* Edited by V. M. Klechkovskii, G. G. Polikarpov, and R. M. Aleksakhin. (A Halsted Press Book.) Pp. xii+371. (Wiley: New York and Toronto; Israel Program for Scientific Translations: Jerusalem and London, 1973.) n.p.

RADIOECOLOGY began during the 1920s with studies of the biological effects of ionising radiation on living organisms in regions with high concentrations of natural radionuclides. By the 1950s it had become clear, at least to the Soviet Union, that the wider environmental effects of increasing radiation levels (especially from atmospheric nuclear testing) were so poorly understood that a vastly enhanced research effort was required. This book, written because the "work done by Soviet scientists has been ignored in published material outside the USSR", reveals just how seriously the Soviet Union takes the problem and how extensive their work has been.

The two fundamental problems in radioecology are to determine how radionuclides migrate within biogeological systems and how ionising radiations affect microorganisms, plants and animals. The 17 chapters describe Soviet researches into the ways that radionuclides (natural and artificial) and radiation behave in soils, forest communities, crops, food chains, natural pastures, wild and farm animals, the far north, water, fish, aquatic plants and man. There is also

a short discussion about the practical problems associated with the measurement of radioactivity in complex natural environments.

Many of these studies have evidently been undertaken with an eye to the consequences of exploiting nuclear energy for peaceful purposes. The editors see nuclear power taking a "prominent place" in the Soviet Union in the near future and envisage the extensive use of nuclear explosions in mining and construction. This being the case, they argue that an understanding of the ecological effects of radioactive pollution must "play an important role in the drafting of suitable restrictions".

Peter J. Smith

## Renewed hope for developmental biology

*Experimental Studies of Amphibian Development.* By E. Hadorn. Pp. vii+138. (Springer-Verlag: Berlin, Heidelberg, New York, 1974.) \$8.20.

THIS short and excellent book is a translation from the German, designed "to introduce the reader to experimental research on development".

Developmental biology is experiencing what Professor Hadorn archly describes as a "Period of Renewed Hope" but many would say that it still remains in a delicate condition. One problem is a growing mass of experimental data that is too flexible and provides unconvincing support for too many theories. Another is that we do not know which organisms to concentrate on. The traditional choice has been the Amphibia, which Hadorn makes the subject of his book. Yet his presentation is far from traditional; his method is to avoid the litigious citation of references and to concentrate on describing a series of clean experiments while omitting the messy ones.

Professor Hadorn guides us through the growth of an individual with experimental analysis as we go along. He mixes deep descriptions of such knotty problems as gastrulation and induction with the light relief of metamorphosis and the mating habits of newts. The book describes many classical experiments and also includes recent discoveries; for instance, we are told that Millipore filters, long used by deluded embryologists as supposed barriers to cell contact, do not act as barriers at all.

The description and diagrams are clear and the subject matter well chosen. In the past, developmental biology has suffered from heavy presentation, but this book offers a delightful contrast.

Peter Lawrence



## Broad visual scope

*The Vertebrate Retina: Principles of Structure and Function.* (A Series of Books in Biology.) By R. W. Rodieck. Pp. x+1044. (W. H. Freeman: San Francisco, 1973.) \$39.50.

ACCORDING to the author this book is an attempt to explain in a simple way, what is known about the organisation and function of the vertebrate retina. That is a formidable task, to which the author devotes over 900 concisely written pages. The coverage is comprehensive, ranging from basic concepts in light and photochemistry, through visual pigments, anatomy and ultrastructure, development, electrophysiology, and behaviour. The result is a real compendium: information and an entry into the literature can be found for topics as diverse as the retinal structure of deep-sea fishes and the characteristics of human colour vision.

It is very unusual to find a book with this breath of cover written by a single individual. The advantages of having only one author are displayed clearly: the plan is coherent, the style of writing is uniform, and repetitions are avoided. There are, of course, also disadvantages. In particular, the coverage is necessarily uneven since it is difficult or impossible for any one individual to be expert in all the fields that are dealt with. In general, I found the coverage of those topics I know little about impressive and very interesting, whereas in those topics with which I am more familiar the treatment seemed rather oversimplified. The treatment of behavioural work with invertebrates seems particularly inadequate. Colour vision in fishes, for example, is dealt with in six lines, including an endorsement of Walls' statement that the best that can be said is that no fish is known not to have colour vision. In fact, there is a great deal of data on that topic, including determinations of wavelength discrimination and spectral saturation functions, and demonstrations of trichromacy, colour mixture, colour contrast, and constancy. Such data are clearly important in view of the great amount of electrophysiological work in that field. It is possible to single out other topics which receive similarly incomplete treatment, but that would be invidious since the general impression is not of the gaps, but of the striking completeness of the description the book gives of vision at the retinal level.

The book is written in a conservative style, presenting a balanced view of the topics and problems raised. Such speculation as occurs is restrained, and is closely attached to experimental data. The book is therefore probably more suited to readers who already have a

reasonable knowledge of vision than it is to, for example, beginning students. The book is without doubt a valuable addition to the literature, and probably represents the most comprehensive review of the whole field that is available at present. References are included up until 1972, so that some of the material is a little out of date, but that is inevitable in a book devoted to a rapidly developing field.

One unusual feature is the complete translation of Ramon y Cajal's monograph on the retina, originally published in 1893, which is included as an appendix. This is, in fact, the second English translation of this work to appear recently, and we should be grateful that after all these years this fundamental study is becoming readily available to English-speaking readers.

W. R. A. Muntz

## Cold avian studies

*Bird Migrations: Ecological and Physiological Factors.* Edited by B. E. Bykhovskii. Pp. v+298. (Halsted, a division of Wiley: New York and Chichester. Israel Program for Scientific Translations: Jerusalem, April 1974.) £12.00.

THIS book is not, as may be expected, a general textbook, but rather a collection of five papers linked together because the work on which they were based was carried out at Rybachy (formerly Rossitten) in the south-eastern Baltic. Paevskii presents detailed lists of ringing recoveries and species maps, which take up two fifths of the book.

Blyumental considers the development of the autumn migratory state based on physiological examinations of 80,000 birds of 15 species. Lyuleeva discusses the energy requirements of swallows in flight during migration. Two short papers by Dol'nik and Gavrilov are on the calorific equivalent of body weight variations in chaffinches and on energy metabolism during flight in some passerines.

It is certainly very useful for those lacking access to the Soviet literature to have these translations. The only pity is that they have to be so expensive. The hard-back format seems to be an unnecessary luxury.

Many western researchers are unaware of the mass of work on bird migration work that has been going on in the Soviet Union. This collection of papers will open their eyes. An even wider deployment of Soviet workers and funds in this general field is at present under way.

One cannot but have reservations about the methods of Lyuleeva. Apart from allowing birds to starve and die in refrigerators, she devised an experiment of profound nastiness. In order to estimate the amount of energy expended in flight, she caught 30 swallows on their nests, weighed them and released them up to 70 km away. To prevent them taking sustenance on the return journey she sewed their beaks shut. She recaptured and reweighed only 17. Any scientist with a spark of decency will be revolted by such unthinking, useless brutality.

G. V. T. Matthews



Chinese bronze sculpture from the middle Chou period (circa 900-600 BC). The patterns represent hair whorls. From *The Husbandry and Health of the Domestic Buffalo*. Edited by W. Ross Cockrill. Pp. xiv+993. (Her Majesty's Stationery Office: London, 1974.) £8.00; £20.00.

## Time for plates of turtle

*Sea Turtles and the Turtle Industry of the West Indies, Florida and the Gulf of Mexico.* Revised edition by Thomas P. Rebel. Pp. 250. (University of Miami: Coral Gables, Florida, March 1974.) \$10.00.

It has often been pointed out that our exploitation of the food resources of the sea remains very largely at the hunting and gathering level. We consume not merely very little of the primary plant production of the sea but very little of the herbivore life too. Mostly, we consume predators further up—sometimes much further up—the food chains, with heavy losses of energy on the way. A book which discusses a group that includes important herbivores is, therefore, welcome.

The aim of this book is practical—the assemblage of the available information necessary “to the proper development and control of our turtle resources”. Dr Rebel starts with the description, identification and distribution of marine turtles, which is accompanied by good figures. Then he reviews what is known of the life history of the six species of turtles found in the western Atlantic and adjacent

seas; these are all the marine turtles known in the world save a seventh species in Australasian waters. This part of the book contains many scattered pieces of information with gaps in between; separate observations have been recorded in disparate fashions. For none of the species of turtle do we have a complete life history, even in outline, but that is no fault of the author's—it reflects the dearth of sustained and methodical observation.

Turtle fishing and the commercial uses of turtles are reviewed and the author describes the progress which has been made in the cultivation of turtles and makes an assessment of the future prospects. A valuable final feature of the book is a bibliography of nearly a hundred pages covering academic as well as practical matters, which is extensively annotated.

Considering the greed with which turtles have been consumed at all stages of their lives with never a thought for the morrow it is astonishing that there is yet time to conserve and exploit them sensibly. Dr Rebel's book gives us reason to believe that turtle populations can be rebuilt and managed, to give a sustainable yield of animal protein and by-products. Let us hope that Dr Rebel will have the reward of seeing this begin to happen in his lifetime.

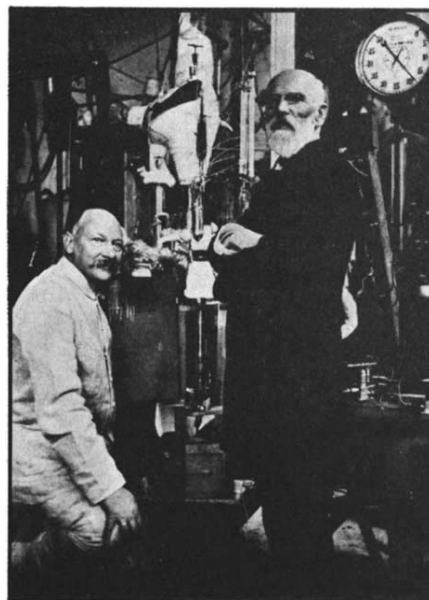
Garth Underwood

### Television:

WITH a programme entitled “The greatest advance since the wheel” *Horizon* (BBC2, November 25, 9.25 p.m.) hit top form yet again. The great advance referred to is superconductivity, which, we were told, is now at the same stage of development as was aviation just before Lindbergh's epic flight. If some similar breakthrough could be achieved with a dramatic development involving superconductivity, then industry would have to recognise the enormous potential offered by developments in the laboratory over the past few years.

Having hit the viewer with this dramatic theme, the programme settled down in slightly more sober vein, and care was taken not to overdramatise achievements already made, nor to underestimate the difficulty of getting industry to provide funds to develop the full potential of superconductivity. As one researcher put it, “you can never convince economists that innovation is worthwhile unless there's a war or crisis”. But this innovation does seem genuinely worthwhile, though I doubt that it can achieve success in all the diverse areas covered in the programme.

Superconducting magnets are, of course, already in use in powerful accelerators and fusion research experiments. Their advantages are that they



J. D. van der Waals (right) and H. Kamerlingh Onnes—pioneers in the field of superconductivity

need scarcely any power to run, just a few kilowatts to keep the helium in the liquid state, and they don't heat up. We were shown prospects of two really big developments: d.c. motors and a.c. generators.

The former is still looked on with suspicion by industry, but seems to have been clasped to the bosom of the

Royal Navy, which is always interested in the prospect of more power from a motor which weighs less. This conjures up some bizarre visions—“it's no use, Sir, we've been hit in the liquid helium bath”—but no doubt such equipment would be no more vulnerable than the complex electronic computers which are already on many modern ships.

As for generating a.c. power with the aid of superconducting coils, industry, it seems, just doesn't want to know. Power generated in experimental setups is tiny by industrial standards: 1 MW, a fraction of 1% of the output of the standard generators used in power stations. The description of research in this area left me slightly puzzled. It seems that the development is so difficult because the superconducting permanent magnet must be made to rotate (cooling helium and all) inside the generator coils. Why, I wonder, do the experimenters not leave the magnet fixed and rotate the coils around it? There is probably a simple explanation, but the point should have been made clear.

There was also a slight puzzle in the otherwise straightforward explanation of superconductivity. According to Bardeen, Cooper and Schrieffer it seems that electrons travel in pairs through a superconductor, and that this prevents them being scattered by impurities in the metal. Perhaps that is what the mathematics tells us, but the physical description and animation offered by *Horizon* was too simplistic and perhaps too naive. Someone could have been on hand to offer more elucidation.

The programme ended as it began, with some more flamboyant stuff, this time about superconducting coils used in magnetic levitation trains. But hard core industrialists would probably have been more intrigued by two more straightforward uses of the new wonder, mentioned earlier in the programme. The powerful magnetic fields produced with the aid of superconductivity can be used to separate weakly magnetic ores from rock which would not otherwise be economically workable; and in a similar way suspended colloidal iron oxide can be removed from water, taking with it just about any impurities which may be present. That offers a better way to treat sewage, to scrub the dirty waters of polluted rivers clean, and so on. And there is no pie in the sky about these straightforward applications of superconductivity.

Perhaps not quite “the greatest advance since the wheel”—it would, incidentally, be interesting to ask a few people what their candidate for this title would be—but good televised science. And that's something to be grateful for.

John Gribbin



# obituary

## Sir Eric Rideal

SIR ERIC RIDEAL, MBE, FRS, the distinguished physical chemist, died on September 25, 1974. He was 84.

From Trinity Hall, Cambridge, Rideal went to Bonn and obtained his PhD there in 1913. After service in the Munitions Inventions Service, he returned to Cambridge in 1920 as Humphrey Owen Jones Lecturer in Physical Chemistry and ten years later became the first Professor of Colloidal Physics. In 1946 he left to be Director of the Davy-Faraday Research Institute and Fullerian Professor at the Royal Institution. He was appointed to the chair of physical chemistry at King's College, London, in 1950. He retired from this post in 1955 and was elected a Fellow in 1963. After his retirement

he continued working at Imperial College, London.

## H. A. Ferreira

HERCULANO DE AMORIM FERREIRA, the Portuguese climatologist, died on May 18, 1974. He was 78.

Ferreira became a professor at the Faculty of Sciences, Lisbon University in 1930 and taught there until 1965. From 1928 to 1937 he also taught at the Portuguese Military Academy and between 1933 and 1934 was visiting professor at Imperial College, London. He was the director of the Geophysical Institute *Infante D. Luis* in Lisbon from 1937 to 1964. In 1943 he was charged with the reorganisation of the National Meteorological Service, which

he directed until 1965. He was an Under Secretary of State for Education from 1944 to 1946.

## J. Chamberlain

JOHN CHAMBERLAIN died on October 11, 1974. He was 37.

He took his doctorate from Imperial College, London in 1962 and was appointed that year to the National Physical Laboratory. There, for the rest of his career, he worked on submillimetre wave Fourier Transform spectroscopy and refractometry. He made important theoretical and practical contributions to precise measurements and was an authority on the high frequency dielectric properties of materials, including solids, liquids, gases and plasmas.

# Announcements

## Appointments

**Bernard Isaacs** has been appointed to the Hayward Chair of Geriatric Medicine at the **University of Birmingham**.

**H. A. Lee** has been appointed to the chair of metabolic medicine at the **University of Southampton**.

**George Edward Mawer** has been appointed to the chair of clinical pharmacology at the **University of Manchester**.

**I. C. Whitfield** has been appointed to the personal chair of neurocommunications at the **University of Birmingham**.

## Awards

**Julian G. Edwards** has been awarded the **1974 Achievement Award of The Worshipful Company of Scientific Instrument Makers** for his development of a Pulsed Energy Laser Monitor.

**Maurice Ewing** has been awarded posthumously the **Penrose Medal of The Geological Society of America**.

**Alfred Edward Ringwood** has been awarded the **Arthur L. Day Medal of The Geological Society of America**.

The Chemical Society is offering an additional award in 1974 which has been sponsored by the British Oxygen Company Limited. The award will be made to the author of the best original paper or papers issued in the past five years (since January 1, 1970) covering the chemistry and/or usage of oxygen. This award is available to men and women of British Nationality, including Commonwealth Citizens or those normally domiciled in the British Isles, and is not limited to Fellows of the Chemical Society.

Nominations and/or applications should be sent to Dr John F. Gibson, The Chemical Society, Burlington House, London W1V 0BN not later than January 31, 1975 and the relevant paper(s) must accompany any such nomination/application.

## Miscellaneous

The **Hollandsche Maatschappij der Wetenschappen** (Dutch Society of Sciences) has the intention of publishing the correspondence of Professor **H. A. Lorentz** (1853–1928), who was Secretary to the Society during the last eight years of his life.

The editing committee would therefore greatly appreciate receiving any information from persons and institutions having in their possession or under

their custody letters written by Professor Lorentz, and to receive copies of these letters with the permission for their publication.

Please address all correspondence to: **Hollandsche Maatschappij der Wetenschappen** (Lorentz Committee), Spaarne 17, Haarlem, The Netherlands.

## International meeting

**January 8, Chemical Properties of Drugs**, London (Dr R. A. Webster, Department of Pharmacology, University College, London WC1).

## Reports and publications

### Great Britain

**Government and the Quality of Life.** By Robert Cooke. Pp. 16. (London: Conservative Publications Centre, 32 Smith Square, SW1, 1974.) [189]

**Information Engineering and Society.** By F. J. M. Laver. (The Ninth Maurice Lubbock Memorial Lecture, 1st March, 1974.) Pp. 18. (London: Oxford University Press, 1974.) 30p net. [199]

**Bulletin of the British Museum (Natural History). Entomology. Supplement No. 22: A Revision of the Old World Genus *Zamarada* (Lepidoptera: Geometridae).** By D. S. Fletcher. Pp. 498 + 123 plates. (London: British Museum (Natural History), 1974.) £35.40. [209]

**Advice to Lecturers: An Anthology Taken from the Writings of Michael Faraday and Lawrence Bragg.** Edited with an introduction by Sir George Porter, F. R. S., and James Friday. Pp. 18 + 4 plates. (London: Mansell Information/Publishing, Ltd., 1974. Published for The Royal Institution.) 85p; \$1.98. [110]

**Practical Implications of a Non-Profit Economy.** (Proceedings of a Symposium co-sponsored by The Sunday Times and The Science Policy Foundation on 26th June, 1974, at the Royal Festival Hall, London. Edited by Maurice Goldsmith. Pp. 59. (London: Science Policy Foundation, Ltd., 1974.) £1.50. [110]

**Bulletin of the British Museum (Natural History). Mineralogy. Vol. 2, No. 8: The Naturally Occurring Chromates of Lead.** By S. A. Williams. Pp. 377–319. £1.95. **Zoology. Vol. 27, No. 4: A Review of *Scotoecus* Thomas, 1910. (Chiroptera: Vespertilionidae).** By J. E. Hill. Pp. 167–188. £1.05. (London: British Museum (Natural History), 1974.) [210]

## Other countries

- Smithsonian Contributions to Zoology, No. 158: Pelagic Studies of Seabirds in the Central and Eastern Pacific Ocean. Edited by Warren B. King. Pp. iv + 277. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$3.70. [29]
- Smithsonian Contributions to Zoology, No. 162: Studies of the Subtribe Tachyina (Coleoptera: Carabidae: Bembidiini), Part II: A revision of the New World-Australian Genus *Pericompsus* LeConte. By Terry L. Erwin. Pp. iv + 96. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) 65 cents domestic postpaid; 45 cents. [29]
- Smithsonian Contributions to Zoology, No. 167: Studies of Neotropical Caddisflies, XVII: The Genus *Smicridea* from North and Central America (Trichoptera: Hydropsychidae) (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$1.50 paper. [29]
- Smithsonian Contributions to Botany, No. 15. Leaf Anatomy and Systematics of New World Velloziaceae. By Edward S. Ayensu. Pp. v + 125. (Washington, DC: Smithsonian Institution Press, 1974. For sale by US Government Printing Office.) \$2.20 Paper. [29]
- United States Department of the Interior: Geological Survey. Water Supply Paper 2017. Improvement of Trout Streams in Wisconsin by Augmenting Low Flows With Ground Water. By R. P. Novitzki. (Prepared in cooperation with Wisconsin Department of Natural Resources. Pp. v + 52. (Washington, DC: United States Government Printing Office, 1973.) 35 cents. (55 cents domestic postpaid.) [29]
- United States Naval Observatory Circular No. 146. Astronomical Data in Machine Readable Form. By Solomon Elvolve. Pp. 11. (Washington, DC: US Naval Observatory, 1974.) [29]
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- La Theorie Des Pulsations Internes de la Terre: Complements. By Georges Dubouardieu. Pp. 47. (Meudon: Laboratoire de Geologie, Avenue Marcellin Berthelot, 92-Meudon, France, 1974.) [39]
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21. Developing muscle-relaxing drugs used in surgery: elucidating the mechanism of the inner ear; work on organo-transition metal chemistry.
- Let Einstein be! restored the status quo.
- It did not last: the Devil howling 'Ho!
- John Collings Squire replied:
20. Alexander Pope wrote it and peared; (k) Owen Seaman.
- (i) David Law Proudfoot; (f) Shakes-Cowley; (h) Edward Baron Thurlow; (f) Andrew Marvell; (g) Abraham Maine (d) Byron; (e) Robert Burns; (c) Sir Henry James Sumner; (b) Charles Chur-
19. (a) Milton; (b) Charles Chur-Chile.
18. Three: one in Australia, two in
17. Poldhu, Cornwall.
18. Uti Geller (see Nature, October
16. Uti Geller (see Nature, October
- glucosidase.
- hexosaminidases A and B; (c) acid α-
15. (a) Acid α-galactosidase; (b) (see Nature, October 4).
- is called Hb Barts hydrops syndrome
- semia I which in its homozygous state
14. Gene deletion causes α-thalas-
13. Paul Berg's committee.
- (c) haem.
12. (a) Chlorophyll; (b) vitamin B<sub>12</sub>;
- paintings.
- It is used for dating wooden panel
11. Dating wood using growth rings.
- catecholamines.
- neuronal transport mechanism for
- receptor blockade and inhibition of
10. Imipramine. Direct α-adreno-
- offending vessel was the Metula.
9. In the Magellan Straits, off the
- Wegener.
8. Continental drift. Alfred
- eye. Ernst Mach.
7. The world as viewed from his left
- Allium cepa.
6. Eat it. It is the common onion,
5. Mr Moon.
4. Queen Victoria.
3. Borodin.
- arrangements of leaves on stems.
- which is commonly found in the
- from Pisa and 'discovered' the series
2. 89. Leonardo Fibonacci hailed
- Edinburgh.
1. S. H. Saker, University of